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ORIGINAL PAPER

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Impact of chemotherapy on antioxidant micronutrient levels in patients with mature lymphoid malignancies

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ABSTRACT

Introduction. Cancer treatments are now intense and are associated with nutritional deficiencies. The nutritional status of a patient may influence the tolerability of chemotherapy.

Aim. We investigated the effects of chemotherapy on serum levels of trace elements (copper, iron, manganese, selenium, and zinc) and vitamins (A, C, and E) in patients with mature lymphoid malignancies (MLMs) at diagnosis and after 3 months.

Material and methods. A case-control study of adults diagnosed with and treated for various MLMs. Thirty-nine cases and 39 age and sex-matched controls were recruited into this study. Venous blood samples were collected from the controls, cases at baseline and after 3 months of chemotherapy. Trace elements were determined by AAS while vitamins were determined by HPLC.

Results. The levels of trace elements and antioxidant vitamins A and E were significantly higher ($p<0.001$) in cases than in controls while vitamin C was lower in cases compared to controls ($p=0.005$). After 3 months of treatment, 28 patients were available for analysis. There was a significant decline ($p<0.001$) in all the levels of trace elements and vitamins after chemotherapy.

Conclusion. Chemotherapy is associated with a significant reduction in antioxidants levels in patients with MLMs.

Keywords. antioxidant micronutrients, chemotherapy, haematologic malignancies

Introduction

Mature lymphoid malignancies are heterogeneous clonal neoplasms of B cells, T cells, and natural killer (NK) cells, which in many respects resemble normal B cells and T cells in stages of differentiation and express the phenotypic markers of mature lymphoid cells. Acute lymphoblastic leukaemias which arise from precursors and express antigenic features of immaturity are excluded.[1,2] Micronutrients are nutrients required by living organisms throughout life in small quantities for a range of physiological functions. Micronutrients include dietary trace elements or minerals and vitamins and other compounds required for normal function in amounts generally less than 100 milligrams/day as opposed to macrominerals which are required in larger quantities.[3] Some of these micronutrients act as antioxidants in the body. Antioxidants are trace elements, vitamins, and enzymes whose combined effect is to neutralize oxidant stress produced from metabolic processes.[4–6]

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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The nutritional status of patients with cancer greatly influences the treatment outcomes.[7]micronutrients, food variety and diet diversity score changed significantly after the induction chemotherapy. No significant relationship was found between the changes in dietary indices and nutritional status. Chemotherapy-related side effects as an additional factor to cancer itself could affect dietary intake of leukaemia patients. The effectiveness of an early assessment of nutritional status and dietary intake should be further investigated in order to deter further deterioration. Deficient diets have detrimental effects on immune status and tolerance of treatment and these patients have poorer quality of life and prognosis. The present approach to the treatment of cancer has become more intensive and aggressive with an associated increase in side effects.[8] Nutrients deficiencies may reduce the response to chemotherapeutic agents, delay the schedule of chemotherapy, and at the same time increase the adverse reactions.

Over the decades, researchers have studied the link between the consumption of food rich in antioxidants and the prevention of degenerative diseases including cancers.[9–13] There have been mixed conclusions with some studies stating a positive effect while others show no effect or even a harmful effect of antioxidant supplementation in certain cancer types.[14–17] However, naturally occurring antioxidants in fruits and vegetables may be better than pharmacological preparations in improving antioxidant status.[18] The concept of the administration of antioxidants during cancer treatment remains a very controversial issue. In general terms, antioxidants could promote or suppress the effectiveness of antitumor treatment and even protect healthy tissues against damage induced by oxidative stress.[4]

Aim

In this study, we investigated the effects of chemotherapy on the levels of antioxidant micronutrients in patients with mature lymphoid malignancies.

Material and methods

Patients

Thirty-nine consecutive patients with MLMs who have not had chemotherapy were recruited. Thirty-nine age and sex-matched controls were also recruited. The diagnoses of MLMs were made based on cytomorphological techniques. Blood smears, bone marrow aspirates, lymph node histology, and immunohistochemistry (where available) were used in making the diagnosis. Each case was reviewed by at least two haematologists at the time of diagnosis. The study participants gave written informed consent.

Before administration of chemotherapy to the patients, 5 milliliters of venous blood were drawn from each patient after 12-14 hours of fasting. Fasting samples were taken to avoid recent dietary influence on measurements of trace elements. This was dispensed into a plain serum bottle. The blood samples were allowed to clot and retract and were centrifuged at 4000 rpm for 15 minutes at room temperature to separate the serum. The serum was dispensed into two plain bottles in aliquots for trace elements and vitamins assays. After 3 months of chemotherapy, another 5 ml of blood was taken from the cases and was processed as above. The serum samples were stored at –80°C until analysis.

Chemotherapy Protocols

Each patient was treated according to the cytomorphological /histological diagnosis. Patients with NHL had either of the following regimens: R-CVP or CHOP in the 21 Day cycle. For CLL patients, the following regimens were used: CVP in a 21 Day cycle or PO Chlorambucil 4mg daily for 2 weeks with 2 weeks resting period in a 28-day cycle. Multiple myeloma patients had CTD in a 28 Day cycle or M+P+Thal) in a 28-42 Day cycle. All patients on Thalidomide received thromboprophylaxis with PO Warfarin 2.5 mg nocte throughout the cycle. Radiotherapy was used in a patient with plasmacytoma. The dose was 3Gy/fraction up to 10 fractions on an alternate day, not exceeding 45Gy. The ABVD regimen was used for patients with Hodgkin lymphoma repeated every 2 weeks. Additionally, patients received general advice on good nutrition same as the premorbid state throughout the study period.

Micronutrient Analysis

The micronutrients (Cu, Fe, Mn, Se, and Zn) in the serum samples were measured using atomic absorption spectrophotometry as described by Kaneko [19] while vitamins A, C, and E were measured by high-performance liquid chromatography using Waters 616/626 (USA) machine.[20] This is part of a study investigating antioxidants and markers of oxidative stress in MLMs.

Ethical considerations

Ethical approval for this study was obtained from the ethical committee of the University of Ibadan/University College Hospital Institution Review Board before the commencement of the study (IRB Research approval number: NREC/05/01/2008a).

Statistical Analysis

Statistical analysis was performed with IBM SPSS for Windows Statistics, version 23. Continuous variables were tested for normal distribution using the Kolmogorov-Smirnov test and the Levene test was used to test the homogeneity of variance. The mean and standard devia-

tions were used to summarize continuous variables such as levels of trace elements and vitamins at baseline and 3 months. An independent sample t-test was used to calculate the difference in means of both groups. In the case of normally distributed variables, a paired-samples t-test was used to compare the means before and after chemotherapy. Mann-Whitney and Wilcoxon signed-rank tests were used for nonparametric variables. Post hoc analyses were used to test for differences between multiple groups means. A p-value < 0.05 was considered statistically significant.

Results

The demographics and social characteristics of the study population were summarized in Table 1. The study population consisted of equal numbers of males (n = 22, 56.4%) and females (n = 17) in both arms. The M: F was 1.2:1 and it was the same for the two groups. The mean (SD) of the age for the cases was 53.5± 16.4 while for the control was 48.2± 14.8, p=0.143. Most of the cases (41%) had tertiary education compared to the control group where all had tertiary education.

Table 1. Demographics and social characteristics of the study population

Variables	Cases (n = 39)	Control (n = 39)
Age	53.5±16.4	48.2±14.8
Sex		
M	22	22
F	17	17
Level of Education		
1. Primary	7	-
2. Secondary	13	-
3. Tertiary	16	35
Occupation		
1. Professionals/ civil servants	15	35
2. Artisans	9	-
3. Students	2	2
4. Trading	6	-
5. Retired	7	2

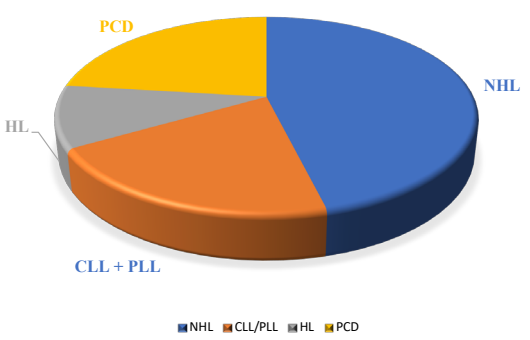

Fig. 1. Proportions of different mature lymphoid malignancies in the study

A total number of 78 participants were included in this study – 39 patients with different mature lymphoid malignancies were recruited and equal numbers of age and sex-matched controls. The cases included 18 patients with NHL, plasma cell dyscrasias (9), CLL (8), Hodgkin Lymphoma (4), this is shown in figure 1. After 3 cycles of chemotherapy, 28 patients were available for analysis.

Assessment of trace elements and antioxidant vitamins in patients with MLMs at diagnosis and control

The serum levels of all trace elements were significantly higher in the cases compared to the controls at diagnosis. The serum concentrations of vitamins A and E were higher in patients with MLMs at presentation relative to the controls. However, the mean level of vitamin C was higher in the controls than in the cases (Table 2).

Table 2. Comparison of serum levels of micronutrients in the controls, cases at diagnosis, and after 3 months of chemotherapy

Parameters	Control n = 39	Before chemotherapy n = 39	3 months PC n = 28
Iron (µg/dl)	111.05 ± 11.5	136.12 ± 13.3#	94.8 ± 1.25¥
Zinc (µg/dl)	94.9 ± 5.9	106.8 ± 6.8#	65.6 ± 6.4¥
Copper (µg/dl)	102.0 ± 11.8	127.8 ± 13.7#	85.3 ± 12.8¥
Manganese (µg/dl)	7.9 ± 0.5	9.16 ± 0.9#	5.5 ± 0.05¥
Selenium (µg/dl)	45.7 ± 5.3	57.4 ± 6.2#	38.4 ± 5.7¥
Vitamin A (µg/dl)	60.62 ± 4.0	63.81 ± 7.9#	33.35 ± 7.23¥
Vitamin C (mg/dl)	1.23 ± 0.1	1.17 ± 0.07*	0.69 ± 0.11¥
Vitamin E (mg/dl)	1.31 ± 0.07	1.54 ± 0.18 #	0.91 ± 0.08¥

PC – post commencement of chemotherapy
- Significantly different from the cases <0.001
* Significantly different from the control <0.005
¥ Significantly different from before chemotherapy <0.001

Effects of chemotherapy on the levels of trace elements and antioxidant vitamins after 3 cycles of chemotherapy

Following chemotherapy, there was a statistically significant reduction (p<0.001 in each case) in serum levels of all trace elements compared to the baseline values. This is shown in Fig 2.

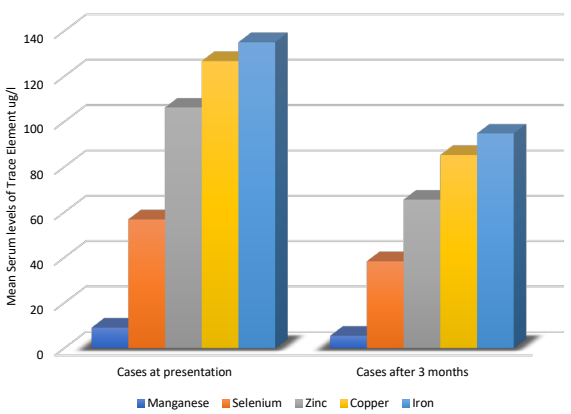

Fig. 2. Levels of trace elements in cases at diagnosis and post 3 months of chemotherapy

A similar trend was seen in all measured vitamins after 3 cycles of chemotherapy as shown in Table 3.

The effects of different chemotherapeutic regimens on trace elements and vitamins

According to Table 3, 8 patients each were treated with CHOP and CVP regimens. Five patients were treated with M+P+Thal, 4 had an ABVD regimen, and 3 were treated with Chlorambucil. The mean (SD) of different analytes were presented in the table below. Post hoc analyses using Scheffe's post hoc criterion for significance indicated that there were no statistical differences in the levels of trace elements, vitamins, and superoxide dismutase using different regimens after 3 months of therapy. The p values for the trace elements were 0.681 for each element, vitamin A (p=0.952), vitamin C (p=0.780), and vitamin E (p=0.680).

Table 3. The effects of different chemotherapeutic regimens on the levels of trace elements and vitamins

	Chemotherapy Regimens					
Parameters	CHOP n = 8	ABVD n = 4	CVP n = 8	M+P+Thal n = 5	Chlorambucil n = 3	p value
Zinc (µg/dl)	67.5±4.0	62.1±4.2	66.5±6.3	67.0±6.3	61.1±13.5	0.681
Copper (µg/dl)	88.9±8.1	78.2±8.4	87.0±12.5	88.1±12.6	76.3±27.0	0.681
Manganese (µg/dl)	5.6±0.3	5.2±0.4	5.5±0.5	5.6±0.5	5.1±1.1	0.681
Selenium (µg/dl)	40.0±3.6	35.2±3.8	39.2±5.6	39.6±5.7	34.3±12.2	0.681
Iron (µg/dl)	98.3±7.9	87.8±8.2	96.5±12.2	97.5±12.2	85.9±26.3	0.681
Vit A (µg/dl)	31.3±12.5	32.7±2.2	35.0±3.3	35.3±3.3	32.2±7.1	0.952
Vit C (mg/dl)	0.7±0.1	0.7±0.1	0.9±0.1	0.6±0.2	0.6±0.2	0.780
Vit E (mg/dl)	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.8±0.2	0.680

CHOP = cyclophosphamide, Doxorubicin, Oncovin, Prednisolone. CVP = cyclophosphamide, Vincristine, Prednisolone. ABVD = Adriamycin, Bleomycin, Vinblastine, Dacarbazine. M+P+Thal = Melphalan, Prednisolone, Thalidomide

Discussion

Trace elements and vitamins play crucial roles in cellular metabolism, cell proliferation, differentiation, and immunological functions. These micronutrients together with antioxidant enzymes and other antioxidants in the cells are important in providing defense against oxidative damage to macromolecules such as DNA. The deficiencies of these nutrients have been associated with the risk of malignancies including MLMs.

In this study, the serum levels of copper were found to be significantly higher in patients than controls at presentation and this agrees with several studies in MLMs.[21–23] The elevated serum copper can be explained by the acute phase reaction on the one hand or it could also be due to malignancy. Copper and its carrier protein caeruloplasmin are positive acute phase reactants that are elevated in inflammatory conditions.[21] There is also evidence that serum copper indicates the extent of disease which is independent of the non-specific acute phase reaction.[24,25] Copper is an essential trace element in many metabolic processes from cellular metabolism to antioxidant defence.[25]

The serum levels of zinc, selenium, iron, and manganese observed in this study were significantly higher in cases than in controls. These observations contradict the established findings that these trace elements are negative acute phase reactants, hence are reduced in inflammatory conditions.[26] Additionally, cancers including MLMs are characterized by malnutrition, especially micronutrient deficiencies resulting from a variety of mechanisms such as cancer-associated anorexia and physiological impairment to digestion and/or absorption.[27] The plausible reason for these observed increases in these trace elements may be due to release from their intracellular locations into the bloodstream as a result of increased cell lysis, which is common in lymphomas and leukaemias. Trace elements such as zinc, selenium, and manganese play roles in protecting the cells from oxidative stress. They are integral parts of enzymes such as SOD and glutathione peroxidase involved in the neutralization of free radicals. Iron serves important functions in cell metabolism, proliferation, and growth.[28] It participates in the Fenton reaction where, in its ferrous state it donates an electron to hydrogen peroxide to form hydroxyl radical, a potent DNA damaging agent, and this may lead to oncogenic activation.[29] The findings in this study agreed with Fahmy et al. who reported significantly elevated Zn and Se in newly diagnosed NHL patients higher than the matched controls. Contrary to our findings, Asfour et al. using the same method of atomic absorption spectrophotometry (AAS) found that zinc was reduced in CLL at diagnosis.[21] Furthermore, Stevens et al. found that selenium was reduced in follicular lymphoma and Hodgkin lymphoma but inductively coupled plasma mass spectrometry method was used in their study.[30] This method is more sensitive than the AAS.

It was found that the serum levels of vitamins A and E were higher in cases than in controls, while vitamin C was lower in cases. The reason for these observations in the serum levels of vitamins A and E in this study is not clear. It is a common practice in the locality of this study for ill patients to use over-the-counter supplements or herbal traditional medicines which may be rich in micronutrients before a definitive diagnosis is made.[31] The serum vitamin C in this study agreed with findings in multiple myeloma patients by Sharma et al and Mehdi et al., but the serum vitamin E levels in this study differed with both studies.[32,33] Both studies demonstrated

that vitamins C and E were lower in multiple myeloma patients than in their controls. It must be pointed out that these studies used colorimetric and fluorometric methods in determining the vitamins. These methods are less specific, accurate, and less reproducible than the HPLC method used in this study.[34] Vitamins are organic compounds required in minute amounts for the proper functioning of cells. There is increasing evidence that antioxidant vitamins can protect against certain types of cancers and other diseases. They can act as the first line in protecting against oxidant stress (vitamin C) or as chain breakers in lipid peroxidation (e.g., vitamin E). Vitamin A is effective in quenching singlet oxygen and inhibits lipid peroxidation.[34] As noted above, micronutrient malnutrition predominates in cancer patients. Furthermore, insufficient supplies of antioxidant vitamins prevalent in many cancers have been linked to increased oxidative stress markers.[8] Combining these two pathophysiological mechanisms, serum vitamins are expected to be lower in cancers. However, the findings in this study did not agree with these hypotheses.

It was demonstrated in this study that after 3 months of chemotherapy that the levels of all trace elements (Cu, Zn, Fe, Se, and Mn) and vitamins (A, C, and E) were significantly reduced with chemotherapy. This can be explained by the fact that patients undergoing chemotherapy and radiotherapy are at increased risk of micronutrient malnutrition, thereby worsening the existing micronutrient negative balance. Most chemotherapeutic agents cause nausea and vomiting, thus significantly reducing food intake. Additionally, intestinal losses of vitamins and electrolytes (metals) in the form of diarrhea are common with chemo-radiotherapy. [27] The degree of these losses will depend on the drug dosage, duration of treatment, excretion rate, metabolism, and individual susceptibility. Another possible explanation relates to increased ROS generated using chemotherapeutic agents such as cyclophosphamide, vincristine, and daunorubicin.[8] It is well established that these agents additionally kill cancer cells through the induction of oxidative stress.[35] In terms of generating oxidative stress, antineoplastic agents are divided into very high level, high level, and low-level producers. Daunorubicin and doxorubicin belong to very high levels, while cyclophosphamide and vincristine (and other vinca alkaloids) belong to high levels and low levels respectively. In this study, no difference was detected using different chemotherapeutic regimens as shown in Table 3. The possible reason for the lack of differences in effects on the levels of trace elements and vitamins could be due to the number of patients in each subgroup and the fact that the regimens used in treating these patients have a combination of agents with different oxidative stress generating potentials in each regimen. The implications of these significant reductions in serum micronutrient levels are impairment of immune cells, recovery, and regeneration of new cells as these processes require micronutrients. Restoration of the physiological levels of these elements may relieve or prevent these impairments. Furthermore, nutrient deficiency may also reduce the response to chemotherapeutic agents, delays in the schedule of chemotherapy, and at the same time increasing adverse reactions.[8]

One of the limitations of this study was the dietary patterns of the study population. The dietary patterns were explored but were met with difficulties because of the lack of standardized methods for assessing dietary intakes and their nutritive contents in the locality of this study. In the absence of validated tools for assessing dietary and the fact that the majority of the participants were drawn from the same ethnic group, it was assumed that they have been exposed to food items with similar nutritive values. Another drawback to the generalizability of the study is the number of patients with mature lymphoid malignancies included in it. A larger study is advocated which will include sufficient numbers of cases with a different diagnosis.

Conclusion

In conclusion, there is a multi-micronutrient deficiency in patients with MLMs undergoing chemotherapy. The strength of this study is that it is perhaps one of the few studies in our locality that assessed the effects of chemotherapy on the antioxidant levels during treatment. These results may guide some cautious interventions in the management of patients with MLMs and other haematological conditions.

References

1. Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. *Blood.* 2008;112(12):4384-4399.
2. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood.* 2016;127(20):2375-2390.
3. Carina L, Susana V, Elisabete C, Manuel G, Miguel B. Micronutrients intake associated with DNA damage assessed by in a human biomonitoring study. *Front Genet.* 2015;6:1-8.
4. Mut-Salud N, Álvarez PJ, Garrido JM, et al. Antioxidant Intake and Antitumor Therapy: Toward Nutritional Recommendations for Optimal Results. *Oxid Med Cell Longev*. 2016;2016:6719534.
5. Khan A, Tania M, Zhang D, Chen H. Antioxidant Enzymes and Cancer. *Chin J Cancer Res.* 2010;22(2):87-92.
6. Shinde A, Ganu J, Naik P. Effect of Free Radicals & Antioxidants on Oxidative Stress : A Review. *J Dent Allied Sci*. 2012;1(2):63-66.
7. Malihi Z, Kandiah M, Chan YM, et al. The effect of dietary intake changes on nutritional status in acute leukaemia patients after first induction chemotherapy. *Eur J Cancer Care (Engl)*. 2015;24(4):542-552.

8. Gröber U, Holzhauer P, Kisters K, Holick MF, Adamietz IA. Micronutrients in Oncological Intervention. *Nutrients*. 2016;8(163):1-30.
9. Ollberding NJ, Maskarinec G, Conroy SM et al. Prediagnostic circulating carotenoid levels and the risk of non-Hodgkin lymphoma: the Multiethnic Cohort. *Blood*. 2012;119(24):5817-5823.
10. Ajila CM, Brar SK. *Role of Dietary Antioxidants in Cancer*. In: Shankar S, Srivastava R. (eds) Nutrition, Diet and Cancer. Springer, Dordrecht, 2012: 377-412.
11. Pal D, Banerjee S. Dietary-induced cancer prevention : An expanding research arena of emerging diet related to healthcare system. *J Adv Pharm Tech Res.* 2012;3(1):16-25.
12. Turati F, Rossi M, Pelucchi C, Levi F, La Vecchia C. Fruit and vegetables and cancer risk : a review of southern European studies. *Br J Nutr*. 2016;113(52):102-110.
13. Chiu BC, Kwon S, Evens AM, Weisenburger DD. Dietary intake of fruit and vegetables and risk of non-Hodgkin lymphoma. *Cancer Causes Control.* 2011;22:1183-1195.
14. Di Pierro F. Antioxidants and cancer : a debate on prevention, progression, hormesis, and cruciferous vegetables. *Nutrafoods.* 2015;14:175-179.
15. Wang L, Sesso HD, Glynn RJ, et al. Vitamin E and C supplementation and risk of cancer in men : posttrial follow-up in the Physicians ' Health Study II randomized trial 1 – 4. *Am J Clin Nutr* 2014;100(3):915-923.
16. Vinceti M, Dennert G, Crespi CM, et al. Selenium for preventing cancer. *Cochrane Database Syst Rev.* 2015;3:1-147.
17. Poljsak B, Milisav I. The Neglected Significance of " Antioxidative Stress ." *Oxid Med Cell Longev*. 2012;2012:1-12.
18. Ames BN. Prevention of mutation, cancer, and other age-associated diseases by optimizing micronutrient intake. *J Nucleic Acids*. 2010;2010:473-479.
19. Kaneko J. *Clinical Biochemistry of Animals*. 4th ed. JJ K, editor. New York: Academic Press Inc; 1999:932.
20. Edem VF, Ige O, Arinola OG. Plasma vitamins and essential trace elements in newly diagnosed pulmonary tuberculosis patients and at different durations of anti-tuberculosis chemotherapy. *Egypt J Chest Dis Tuberc.* 2015;64(3):675-679.
21. Asfour IA, Hegab HM, Mohammed RM, et al. Assessment of copper, zinc and nitric oxide status in patients with chronic lymphocytic leukemia. *Can Res Metastasis.* 2017;1(2):3-8.
22. Abdelgawad MI, Mohammed HSE. Overview of Angiogenesis in Upper Egypt Open Hodgkin's Lymphoma Patients. *Qual Prim Care*. 2016;24(3):125-132.
23. Mohammad-Hassan KA, Asoudeh M, Hosein Fallahi Kord G, et al. Copper and zinc in stage I multiple myeloma: relation with ceruloplasmin, lipid peroxidation, and superoxide dismutase activity. *Horm Mol Biol Clin Investig.* 2018;37(3):1-6.
24. Akanni EO, Onuegbu AJ, Adebayo TO, Eegunranti BA, Oduola T. Assessment of Some Selected Trace Metals in Chronic Myeloid Leukemia Patients in a Tertiary Health Facility in South West Nigeria. *Asian J Med Sci.* 2013;5(4):71-75.
25. Kaiafa GD, Saouli Z, Diamantidis MD, et al. Copper levels in patients with hematological malignancies. *Eur J Intern Med.* 2012;23:738-741.
26. Galloway P, Mcmillan DC, Sattar N. Effect of the inflammatory response on trace element and vitamin status. *Ann Clin Biochem*. 2000;37:289-297.
27. Ströhle A, Zänker K, Hahn A. Nutrition in oncology : The case of micronutrients. *Oncol Rep*. 2010;24:815-828.
28. Zhang C, Zhang F. Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities. *Protein Cell*. 2015;6(2):88-100.
29. Bystrom LM, Guzman ML, Rivella S. Iron, and ROS : Friends or Foes of Cancer Cells? *Antioxid Redox Signal*. 2014;20(12):1917-1924.
30. Stevens J, Waters R, Kassam S, et al. Serum selenium concentration at diagnosis and outcome in patients with haematological malignancies. *Br J Haematol.* 2011;154:448-456.
31. Edem VF, Ige O, Arinola OG. Plasma vitamins and essential trace elements in multi-drug resistant tuberculosis patients before and during chemotherapy. *Egypt J Chest Dis Tuberc.* 2016;65(2):441-445.
32. Sharma A, Tripathi M, Satyam A, Kumar L. Study of antioxidant levels in patients with multiple myeloma. *Leuk Lymphoma.* 2009;50(5):809-815.
33. Mehdi WA, Zainulabdeen JA, Mehde AA. Investigation of the Antioxidant Status in Multiple Myeloma Patients : Effects of Therapy. *Asian Pacific J Cancer Prev.* 2013;14(6):3663-3667.
34. Shenkin A, Roberts NB. Vitamins and Trace Elements. In: Carl A. Burtis ERA, Bruns DE, editors. *Tietz Textbook of Clinical Chemistry And Molecular Diagnostics.* 5th ed. 3251 Riverport Lane St. Louis, Missouri 63043: Elsevier; 2012:895-983.
35. Conklin KA, Nicolson GL. Molecular Replacement in Cancer Therapy: Reversing Cancer Metabolic and Mitochondrial Dysfunction, Fatigue and the Adverse Effects of Cancer Therapy. *Curr Cancer Ther Rev.* 2008;4(1):66-76.

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ORIGINAL PAPER

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Diffusion weighted imaging in differentiation of the clear cell RCC from the major non-clear cell RCC subtypes

Department of Radiology, University of Medical Sciences, Prof Dr Cemil Tascıoglu City Hospital, İstanbul, Turkey

ABSTRACT

Introduction. Renal cell carcinoma (RCC) is the most common malignant renal tumor in adults accounting for 80-90% of primary malignant adult renal neoplasms. RCC represents a very heterogenous groups of tumors with a number of distinct histological varieties, of which the major 3 subtypes are clear cell RCC (70-80%), papillary RCC (13-20%), and chromophobe RCC (5%). Imaging features are varied from solid and relatively homogenous appearance to markedly heterogenous appearance with cystic changes, hemorrhage and necrosis. The use of diffusion weighted imaging (DWI) for RCC subtyping and also for differentiation of high grade and low grade tumors has been showed to be useful in many studies in the literature.

Aim. In this study, we aimed to determine the comparative contribution of DWI in differentiation of the clear cell RCC from the major non-clear cell RCC subtypes at standard high b-value (1000 s/mm^2) versus low b-value (500 s/mm^2). In addition, we also aimed to assess the diagnostic performance of DWI for differentiating high grade clear cell RCC from low grade clear cell RCC based on Fuhrman grades in our patients.

Material and methods. 62 cases with a prediagnosis of RCC according to MRI findings including DWI sequence with histological verification and subtyping of renal cortical tumor following a total or partial nephrectomy were included in the study.

Results. Among 62 cases, 46 were male and 16 were female, with mean age of 59.5±15.7. Pathological diagnoses of 62 cases were as follows, clear cell RCC, (44) papillary cell RCC (14) and chromophobe cell RCC (4). They were divided into two groups as clear cell RCC group (44 cases) and non-clear cell RCC group (18 cases). There was no statistically significant difference between the mean ADC values of clear cell and non-clear cell groups at b-value of 1000 s/mm^2 ($p>0.05$). However, the mean ADC level for clear cell RCC group at b-value of 500 s/mm^2 were significantly higher than for non-clear cell RCC group ($p<0.05$). When a value of 0.99x10^{-3} mm^2/s was set as cut-off for ADC at b-factor of 500 s/mm^2, differentiation was achieved with a high sensitivity (91%) and specificity (56%). Regarding the diagnostic performance of DWI for differentiating high from low Fuhrman grades clear cell RCCs, there was no statistically significant difference between the ADC values of Grade I-II clear cell RCC cases and Grade III-IV clear cell RCC cases at b-factor of 1000 s/mm^2 ($p>0.05$). However, ADC values for grade III-IV group was statistically significantly lower than ADC values for Grade I-II group at b-factor of 500 s/mm^2 level.

Conclusion. ADC measurements at moderate b-value of 500 s/mm^2 were more sensitive in subtyping and grading of RCC cases. This technique can be used in clinical practice as a fast and additional sequence in abdominal MRI.

Keywords. apparent diffusion coefficient, diffusion weighted imaging, renal cell carcinoma

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Introduction

Renal cell carcinoma (RCC) is the most common malignant renal tumor in adults accounting for 80-90% of primary malignant adult renal neoplasms.[1,2] The age of presentation is typically 50-70 years and there is a moderate male predilection of 2:1.[3,4] Macroscopic hematuria, flank pain and palpable flank mass are typical clinical findings.[5] However, due to the widespread use of imaging, incidental detection of asymptomatic lesions have been increased. RCC represents a very heterogenous groups of tumors with a number of distinct histological varieties, of which the major 3 subtypes are clear cell RCC (70-80%), papillary RCC (13-20%), and chromophobe RCC (5%).[1,6] Imaging features are varied from solid and relatively homogenous appearance to markedly heterogenous appearance with cystic changes, hemorrhage and necrosis. The main forms of RCC subtypes can often be non-invasively differentiated by imaging characteristics on dynamic contrast enhanced MRI. While clear cell RCC is typically heterogenous secondary to necrosis, cystic change or hemorrhage, and has high signal intensity on T2w images atributable to clear cytoplasms of large uniform cells, most papillary RCCs show low signal intensity on T2w MRI. Chromophobe RCCs may have a homogeneous solid appearance even when large. On dynamic contrast-enhanced images, while clear cell RCCs are highly vascular masses, papillary RCCs are hypovascular and chromophobe RCCs may exhibit a central stellate scar and spoke-wheel enhancement.[6] In addition to that, the use of diffusion weighted imaging (DWI) for RCC subtyping and also for differentiation of high grade and low grade tumors has been showed to be useful in many studies in the literature.

Aim

In this study, we aimed to determine the comparative contribution of DWI in differentiation of the clear cell RCC from the major non-clear cell RCC subtypes which are papillary RCC and chromophobe RCC at standard high b-value (1000 s/mm^2) versus low b-value (500 s/mm^2). In addition, we also aimed to assess the diagnostic performance of DWI for differentiating high grade clear cell RCC from low grade clear cell RCC based on Fuhrman grades in our patients.

Material and methods

Patients

62 cases with a prediagnosis of RCC according to MRI findings including DWI sequence with histological verification and subtyping of renal cortical tumor following a total or partial nephrectomy between February 2011 and November 2012 were included in the study. The study was approved by the institutional ethics committee and written informed consent was taken from all patients prior to be included in the study.

Sample cases are presented below (Figure 1-3).

MRI technique

Conventional MRI and DWI examinations were performed with Siemens Avanto 1.5 Tesla MR scanner (Siemens Erlangen, Germany). DWI echo-planar images (TR: 4000, TE: 76, FOV of 400 mm, matrix: 156 x 192, NEX: 3, sectional thickness: 5 mm with a 1 mm intersection gap) were obtained in the axial plane before the contrast administration. DWI were obtained by diffusion gradients between, 500 and 1000 s/mm^2 b-values. An ADC map was automatically constructed in the workstation. Mean ADC values of all lesions were automatically measured by using the ADC maps according to the formula ADC = (lnS0 – lnS)/b (signal intensity values are measured as S0 at b = 0 s/mm^2, b=500 s/mm^2, at b = 1000 s/mm^2).

ADC measurement

Measurements were performed by placing a region of interest (ROI) of 1 cm diameter on the solid parts that enhances in postcontrast images and shines in DWI images. The ROI did not include normal parenchymal tissue, or haemorrhagic or necrotic areas The lowest one of three consecutive measurements were included in the analyses.

Statistical analysis

Differences of ADCs of lesions and normal parenchyma were assessed with paired samples t-test. Differentiability of clear-cell RCC from non-clear cell RCC by ADC values was evaluated by ROC curve analysis. Moreover, a mean of 3 different ADC values from normal renal parenchyma were taken for comparing with the ADC values of lesions. A p value < 0.05 was considered as statistically significant.

Results

62 cases, of whom 46 were male and 16 were female, with an age range between 26 to 86 years (mean 59.5±15.7 years) were recruited for the study. Pathological diagnoses of 62 cases were as follows, 44 cases of clear cell RCC, 14 cases of papillary cell RCC and 4 cases of chromophobe cell RCC. They were divided into two groups as clear cell RCC group (44 cases) and non-clear cell RCC group (18 cases). There was no statistically significant difference between the mean ADC values of clear cell and non-clear cell groups at b-value of 1000 s/mm^2 ($p>0.05$) (Table 1).

However, the mean ADC level for clear cell RCC group at b-value of 500 s/mm^2 were significantly higher than for non-clear cell RCC group ($p<0.05$). When a

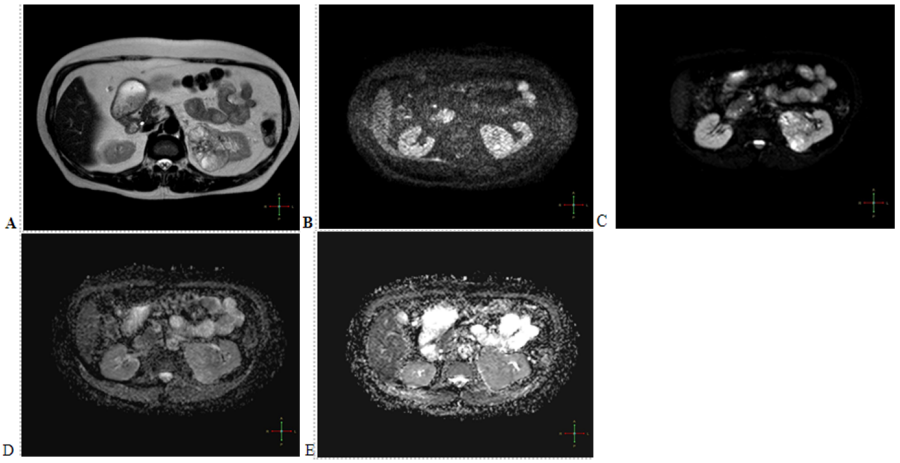

Fig. 1. Case 1: Clear cell RCC in a 56 years-old male patient: (A) heterogeneous hyperintense lesion with cystic necrosis fields in T2WI of left kidney; (B-C) restriction in diffusion series; (D-E) b-factor 1000 s/mm^2 ADC: 1.68x10^{-3} mm^2/s, b-factor 500 s/mm^2 ADC: 2.05x10^{-3} mm^2/s.

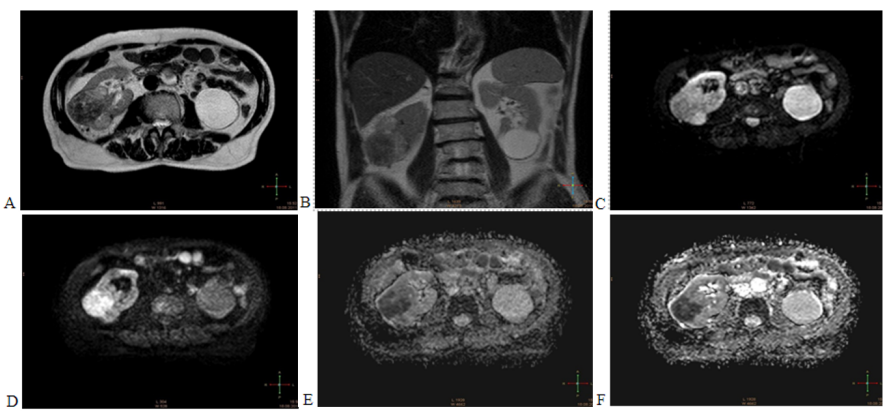

Fig. 2. Case 2: Papillary cell RCC in right kidney, and simple cyst in left kidney of an 80 years-old male patient. (A-B) hypointense heterogeneous lesion with exophytic extent in right kidney, and hyperintense cystic lesion with exophytic extent from left kidney in T2WI; (C-D) restriction in solid lesion at right kidney in diffusion series (T2 shining effect on simple cyst in left kidney at b-factor of 1000 s/mm^2); (E-F) b-factor 1000 s/mm^2 ADC: 0.61x10^{-3} mm^2/s, b-factor 500 s/mm^2 ADC: 0.93x10^{-3} mm^2/s for lesion in right kidney, and b-factor 1000 s/mm^2 ADC: 2.53x10^{-3} mm^2/s, b-factor 500 s/mm^2 ADC: 2.79x10^{-3} mm^2/s for cyst in left kidney

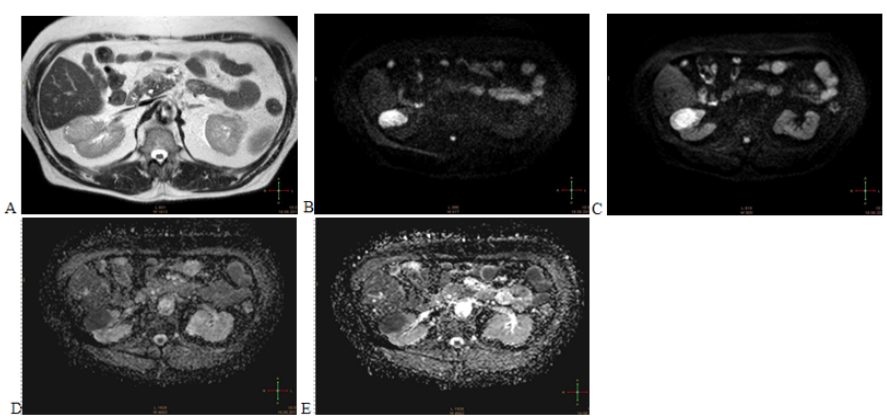

Fig. 3. Case 3: chromophob cell RCC in right kidney of a 63 years-old male patient. (A) heterogeneous hypointense lesion in T2WI of right kidney; (B-C) overt restriction in lesion in diffusion series; (D) b-factor 1000 s/mm^2 ADC: 0.68x10^{-3} mm^2/s, (E) b-factor 500 s/mm^2 ADC: 0.94x10^{-3} mm^2/s.

Table 1. RCC subtypes

	Clear cell (n=44)		Non-clear cell (n=18)		p
	Mean	SD	Mean	SD	
ADC at b-factor of 1000 s/mm²	1.292	0.371	1.017	0.388	0.117
ADC at b-factor of 500 s/mm²	1.536	0.421	1.129	0.389	**0.030***
NP of ADC at b-factor of 1000 s/mm²	1.893	0.202	2.000	0.080	**0.017***
NP of ADC at b-factor of 500 s/mm²	2.214	0.278	2.276	0.195	0.727

**: p<0.05*

Difference between ADC values of clear cell and non-clear cell groups at b-factor of 1000 s/mm² was not statistically significant (p>0.05).

ADC values of clear cell group at b-factor of 500 s/mm² were significantly higher than the non-clear cell group (p<0.05).

Table 2. Fuhrman grades in clear-cell subtype

	I-II clear cell (n=24)		III-IV clear cell (n=20)		p
	Mean	SD	Mean	SD	
ADC at b-factor of 1000 s/mm²	1.413	0.363	1.147	0.343	0.075
ADC at b-factor of 500 s/mm²	1.738	0.383	1.293	0.338	**0.018***
NP of ADC at b-factor of 1000 s/mm²	1.918	0.236	1.864	0.161	0.643
NP of ADC at b-factor of 500 s/mm²	2.241	0.254	2.181	0.314	0.792

**: p<0.05*

In cases with a clear-cell pathology; difference between ADC values of grade I-II clear cell and grade III-IV clear cell groups at b-factor of 1000 s/mm² was not statistically significant (p>0.05).

In cases with a clear-cell pathology; ADC values of grade I-II clear cell group at b-factor of 500 s/mm² were significantly higher than the grade III-IV cell group (p<0.05).

value of $0.99x10^{-3}$ mm²/s was set as cut-off for ADC at b-factor of 500 s/mm², differentiation was achieved with a high sensitivity (91%) and specificity (56%). Regarding the diagnostic performance of DWI for differentiating high from low Fuhrman grades clear cell RCCs, there was no statistically significant difference between the ADC values of Grade I-II clear cell RCC cases and Grade III-IV clear cell RCC cases at b-factor of 1000 s/mm² (p>0.05). However, ADC values for grade III-IV group was statistically significantly lower than ADC values for Grade I-II group at b-factor of 500 s/mm² level (Table 2).

Discussion

RCC which is the most frequent malignant renal tumor in adults arises from tubular epithelium and represents a number of distinct histological variants associated with different metastatic potential, prognosis and management. Many studies showed that as compared to clear cell RCC, chromophobe and papillary RCC have better prognoses and may be treated with kidney sparing approaches rather than radical nephrectomy.[7] Therefore, preoperative discrimination of clear cell RCC from non-clear cell subtypes is very important. DWI is a method that provides characterization of biological tissues based on irregular diffusion motion of water molecules, which can be integrated easily into a conventional MRI providing more detailed information at the cellular. The diffusion of free water in tissues is restricted basically by cell membranes and therefore, by the increased cellularity of the tissue, as in tumoral processes. [8] Diffusion weighted images are obtained from T2w images with the addition of diffusion weighting gradient, that is the b-value which shows the extent to which the sequence is sensitive to the diffusion.[9] ADC is the mathematical expression of diffusion as a result of marking the signal loss on the map, which occurs after applying diffusion gradient. The ADC value of free water molecules at 37°C is reported to be 3.0×10^{-3} mm²/s. This value is taken as the reference point, and it is the value at which the diffusion is maximum. When there is restriction to diffusion, the ADC value decreases.[8] When the b value is low, the diffusion weight of the sequence decreases and it is affected by T2 time and perfusion. The perfusion of malignant tumours is markedly higher than that of the benign ones, and so at low b values the ADC of malignant lesions are measured as higher than it is artifactually from capillary perfusion effect.[10] It has been postulated that the b-value gradients between 0 and 200 s/mm² primarily measure perfusion, whereas gradient strengths greater than 200 s/mm² primarily measure diffusion.[11,12] In a study performed for discriminating malignant from benign vertebral compression fracturesMore pronounced difference in the mean ADC value in the group of low b-value (<500) than in the group of b-value (≥500) was reported. The authors concluded that, low b-value (<500) is a more valuable parameter than standard b-value (≥500) to show mean

ADC differences.[13] Wang et al. tried to determine the subtypes of 85 RCCs observed in 83 patients (49 clear cell tumors, 22 papillary tumors and 14 chromophobe tumors) at 3T MRI at b-value of 500 and 800 s/mm^2. They found that papillary RCCs (1.1 x 10^{-3} mm^2/s) and chromophobe RCCs (1.3 x 10^{-3} mm^2/s) had significantly lower mean ADC values than clear cell RCCs (1.8 x 10^{-3} mm^2/s).No significant differences were found between papillary and chromophobe RCCs. However, at b-value of 800 s/mm^2, they could be able to differentiate all subtypes, and concluded that this b-value is more sensitive.[14] On the other hand, in a meta analysis conducted to determine the comparative diagnostic performance of standard b-value (800–1000 s/mm^2) versus low b-value (400–500 s/mm^2) DW-MRI in the detection of RCC. The study concluded that the standard b-value DW-MRI showed a superior specificity but an approximately equivalent sensitivity to low b-value DW-MRI in detecting RCC. However, low b-value DW-MRI displayed an overall superior diagnostic accuracy over standard b-value DW-MRI.[15] Because, when the b value increases the spatial resolution decreases significantly, and when the b value decreases then the perfusion effect appears, in our study the b value was taken as 500 and 1000 s/mm^2. Similar to this study, significant difference in terms of mean ADC value between clear cell and non-clear cell groups was not observed when b-value was chosen as 1000 s/mm^2, but ADC level of non-clear cell group was significantly lower at b-value of 500 s/mm^2 in our study. Although it was reported that for the b value equal to or higher than 600 s/mm^2, the effect of perfusion is minimal and can be ignored, the appearance of significant difference between two groups when the b-value was decreased to 500 s/mm^2, in our study can be explained to some extent by explicitness of perfusion effect.[16] In addition, the papillary cell RCC group had the lowest ADC values in our patients, as in the study of Wang et al. and Taouli et al. [14,17]

In a study conducted to assess the value of DWI in differentiating the various subgroups of renal masses, it was found that ADC values higher than 2.12x10^{-3} mm^2/s were only observed in low-grade cancers, and ADC values lower than 1.50x10^{-3} mm^2/s were only observed in high-grade cancers.[17] In various studies, it was also found that high grade clear cell RCCs have significantly lower mean ADC values than low grade clear cell RCCs.[10,14,18] In our study, we also tried to discriminate the preoperative histological grades of clear cell RCC cases. These tumors were graded as Fuhrman grade I-II (low-grade) and Fuhrman grade III-IV (high-grade) according to postoperative pathological findings. There was also no significant difference between groups at b-value of 1000 s/mm^2, but there was a statistically significant difference when b-value was selected as 500 s/mm^2. ADC values at b-value of 500 s/mm^2 were lower in Fuhrman grade III-IV clear cell RCC cases, when compared with Fuhrman grade I-II group. This finding can be explained by explicit limiting in diffusion due to increased cellularity.

The main limitations of our study includes the limited number of patients and the validity of the cut-off ADC value only for the imaging protocols performed with the b values defined for our MRI device.

Conclusion

ADC measurements at moderate b-value of 500 s/mm^2 were more sensitive in subtyping and grading of RCC cases. This technique can be used in clinical practice as a fast and additional sequence in abdominal MRI. Completion period is as short as 17 seconds, and it can provide both qualitative and quantitative findings for diagnosis.

References

1. Ng CS, Wood CG, Silverman PM, et al. Renal cell carcinoma: diagnosis, staging, and surveillance. *AJR Am J Roentgenol.* 2008;191(4):1220-1232.
2. Sheth S, Scatarige JC, Horton KM, et al. Current concepts in the diagnosis and management of renal cell carcinoma: role of multidetector ct and three-dimensional CT. *Radiographics.* 2001;21(1):237-254.
3. Federle MP, Jeffrey RB, Woodward PJ, et al. *Diagnostic Imaging: Abdomen.* Amirsys®, Lippincott Williams & Wilkins; 2009. ISBN:1931884714.
4. McPhee SJ, Papadakis MA. *Current Medical Diagnosis and Treatment 2009.* McGraw-Hill Professional; 2008. ISBN:0071591249.
5. Hollingsworth JM, Miller DC, Daignault S, Hollenbeck BK. Rising incidence of small renal masses: a need to reassess treatment effect. *J Natl Cancer Inst.* 2006;98:1331-1334.
6. Oliva MR, Glickman JN, Zou KH, et al. Renal cell carcinoma: t1 and t2 signal intensity characteristics of papillary and clear cell types correlated with pathology. *AJR Am J Roentgenol.* 2009;192(6):1524-1530.
7. Hötker A, Mazaheri Y, Wibmer A, et al. Use of DWI in the Differentiation of Renal Cortical Tumors. *American Journal of Roentgenology.* 2016;1:100-105.
8. Woodhams R, Ramadan S, Stanwell P, et al. Diffusion-weighted imaging of the breast: principles and clinical applications. *Radiographics.* 2011.31:1059-1084.
9. Li Y, Chen Z, Wang J. Differential diagnosis between malignant and benign hepatic tumors using apparent diffusion coefficient on 1.5-T MR imaging: a meta analysis. *Eur J Radiol.* 2012;81:484-490.
10. Rosenkrantz AB, Niver BE, Fitzgerald EF, Babb JS, Chandarana H, Melamed J. Utility of the apparent diffusion coefficient for distinguishing clear cell renal cell carcinoma of low and high nuclear grade. *AJR Am J Roentgenol.* 2010;195:W344-351.

11. Iima M, Le Bihan D. Clinical intravoxel incoherent motion and diffusion MR imaging: past, present, and future. *Radiology.* 2016;278:13-32.
12. Kim HS, Suh CH, Kim N, Choi CG, Kim SJ. Histogram analysis of intravoxel incoherent motion for differentiating recurrent tumor from treatment effect in patients with glioblastoma: initial clinical experience. *AJNR Am J Neuroradiol.* 2014;35:490-497.
13. Luo Z, Litao L, Gu S, et al. Standard-b-value vs low--b-value DWI for differentiation of benign and malignant vertebral fractures: a meta-analysis. *Br J Radiol.* 2016;89(1058):20150384.
14. Wang H, Cheng L, Zhang X, et al. Renal Cell Carcinoma: Diffusion-weighted MR Imaging for Subtype Differentiation at 3.0 T. *Radiology*. 2010;257:135-143.
15. Tang Y, Zhou Y, Du W, et al. Standard b-value versus low b-value diffusion-weighted MRI in renal cell carcinoma: a systematic review and meta-analysis. *BMC Cancer.* 2014;14:843.
16. Baron P, Dorrius MD, Kappert P, et al. Diffusion-weighted imaging of normal fibroglandular breast tissue: influence of microperfusion and fat suppression technique on the apparent diffusion coefficient. *NMR Biomed.* 2010.23:399-405.
17. Taouli B, Thakur RK, Manelli L, et al. Renal lesions: characterization with diffusion-weighted imaging versus contrast-enhanced MR imaging. *Radiology.* 2009;251:398-407.
18. Goyal A, Sharma R, Bhalla AS, Gamanagatti S, Seth A, Iyer VK, Das P. Diffusion-weighted MRI in renal cell carcinoma: a surrogate marker for predicting nuclear grade and histological subtype. *Acta Radiol.* 2012;53:349-358.

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ORIGINAL PAPER

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Randomized, open-label, controlled, comparative clinical study to evaluate the safety and efficacy of Pilex Forte tablets in combination with Pilex Ointment application for the effective management of common ano-rectal conditions

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ABSTRACT

Introduction. Haemorrhoids and anal fissures are considered as the most common anorectal conditions.

Aim. To evaluate the safety and efficacy of Pilex Forte tablets in combination with Pilex Ointment as compared to the Standard of Care in common anorectal conditions

Material and methods. Randomized, open-label, controlled comparative clinical study conducted on 162 patients of either sex, aged between 18-50 years, confirmed with common anorectal conditions. As per randomization, patients received either standard of care or Pilex Forte tablet along with Pilex Ointment at a recommended dose of two tablets twice daily and twice daily local application (anal) for a period of 4 weeks. Clinical safety and efficacy assessments were carried out at study specific scheduled visits. Laboratory assessments were carried out only at screening and at the end of the study.

Results. Patients who completed the study were considered for statistical analysis. Significant clinical improvement was observed in patients with anorectal conditions who received Pilex Forte tablet and Pilex Ointment than to those who received standards of care. No abnormal lab values were recorded and there were no adverse events reported during the study period.

Conclusion. Pilex Forte tablet along with Pilex Ointment at recommended dose is safe and effective in the management of anorectal conditions like haemorrhoids and fissure-in-ano.

Keywords. anorectal, fissure-in-ano, haemorrhoids

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Introduction

Haemorrhoids and anal fissures (fissure – in – ano) are considered as the most common anorectal conditions. Although; they are not life threatening, they can detrimentally impact patients' quality of life and daily living activities.[1] Anorectal disorders are commonly seen by both primary and specialty health care providers such as gastroenterologists. Nonsurgical anorectal management has become an increasingly emphasized portion of the core curriculum for many gastroenterology training programs.[2]

Haemorrhoids are abnormalities of submucosal, venous structures in the anorectum resulting in symptoms.[3] The prevalence of symptomatic haemorrhoidal disease is difficult to evaluate due to a high percentage of people with symptomatic haemorrhoids who avoid evaluation or those who successfully use lifestyle modifications, non-pharmacological therapies, or over the counter agents for their symptom relief. It has been estimated that up to 75% of individuals have symptomatic haemorrhoids at least once in their lifetime.[4] The exact prevalence is unknown because most patients are asymptomatic and do not seek care from a physician. A study shown that patients undergoing routine colorectal cancer screening found 39% prevalence of haemorrhoids, with 55% of those patients reporting no symptoms.[5]

Haemorrhoids usually develop when the venous drainage of the anus is changed, causing the venous plexus and connecting tissue to dilate, producing an outgrowth of anal mucosa from the rectal wall. Haemorrhoids are classified depending on their location. Haemorrhoids which are distal to the dentate line are considered external and are covered by sensate squamous epithelium. Internal haemorrhoids are situated proximal to the dentate line and are covered by insensate columnar epithelium. As per Golighter classification, internal haemorrhoids are classified into four degrees: First-degree internal haemorrhoids do not prolapse into the anorectum; second-degree haemorrhoids prolapse into the anorectum and spontaneously reduce; third-degree haemorrhoids prolapse into the anorectum and require manual reduction; and fourth-degree are unable to be reduced manually.[4]

Symptoms depend upon the location of haemorrhoids. External haemorrhoids are characteristically asymptomatic but may cause pain due to their overlying sensate squamous mucosa, predominantly when thrombosis occurs. The most common symptom of internal haemorrhoids is painless rectal bleeding.[6] The treatment of haemorrhoids involves lifestyle modification, medical, and surgical approaches[7-8]. First-line conservative treatment of haemorrhoids consists of a high-fiber diet, (sitz) baths, increased water intake, warm water, and stool softeners.[9-10] Non-operative treatment includes Sclerotherapy, Rubber band ligation, Cryotherapy, Infrared coagulation, Radiofrequency ablation.[11]

Anal fissures are tears that occur beneath the dentate line in the anal canal. Anal fissures are classified as acute or chronic depending on their time course with those lasting 6 weeks or longer being classified as chronic.[12] The etiopathogenesis of anal fissures is multifactorial. Manometric studies have revealed that augmented resting anal pressure is common in patients diagnosed with anal fissures. Majority of fissures occur in the posterior midline and are solitary. Fissures occurring away from the midline or fissures that fail to heal should prompt additional evaluation for alternative etiologies such as Crohn's disease, tuberculosis, HIV, other sexually transmitted diseases or malignancy.[13] Over half of simple, acute anal fissures resolve without specific medical therapy. Fissures that fail to resolve spontaneously should receive medical therapy. Dietary and lifestyle changes are the important aspects of the non-surgical care of anal fissures. The primary goal of medical therapy is to relax the anal sphincter and allow the fissure to heal through interruption of the sphincter spasm and tearing cycle. Warm sitz baths are also recommended based on studies that have revealed that they can help to briefly decrease internal and external sphincter spasticity and decrease pain.[14] Bulking agents has been revealed in well-designed studies to be beneficial in this ailment.[15]Among the variety of agents that have been investigated to chemically relax the anal sphincter, topical nitroglycerin Ointment has been most extensively studied in the treatment of anal fissure with success rate of 80%.[15]

Pilex Forte tablet is a polyherbal formulation consisting of principal ingredients like *Balsamodendron mukul* (Purified), Shilajeet (Purified), *Melia azadirachta*, *Berberis aristata, Emblica officinalis, Terminalia chebula, Terminalia belerica, Cassia fistula, Bauhinia variegata,* and *Mesua ferrea.* Pilex Ointment is a herbo-mineral formulation consisting extracts of *Mimosa pudica, Eclipta alba, Vitex negundo, Calendula officinalis, Mimosa pudica, Eclipta alba, Vitex negundo, Calendula officinalis, Cinnamomum camphora, Tankana, Yashada bhasma* which have anti-inflammatory, analgesic, wound healing, membrane stabilizing, anti-microbial and antioxidant actions. Therefore, a safety and efficacy study was planned to further evaluate the role of Pilex Forte along with local application of Pilex Ointment as compared to standard of care in the management of common ano-rectal conditions like haemorrhoids and fissure-in-ano.

Aim

To evaluate the safety and efficacy of Pilex Forte tablets along with Pilex Ointment in the treatment of common ano-rectal conditions in comparison with the standard of care.

Material and methods

This study was a Randomized, Prospective, Interventional, Open-label, Controlled Comparative clinical study conducted at State Indian System of Medicine (ISM) Department, Department of Surgery and Department of Clinical Pharmacology, JIPMER, Puducherry. All essential study documents were submitted to the JIPMER Institutional Ethics committee for review and approval with the vide approval number JIP/IEC/2018/050. All the patients gave their informed consent for their active participation in the study.

This was a randomized, prospective, interventional, open-label, controlled comparative clinical study conducted on 162 patients of either sex, aged between ≥18 to ≤50 years clinically confirmed with the history of common anorectal conditions like haemorrhoids and Fissure-in-Ano. Common ano-rectal conditions include; Haemorrhoids (symptoms of Grade I and Grade II Haemorrhoids, i.e, Haemorrhoids which may protrude out of anal canal during defecation, but reduce spontaneously) with symptoms like Pain, Itching, Bleeding, protruding mass per anus, difficulty in passing stools and constipation. Fissure-in ano [Chronic or recurrent fissures (>4 months] with symptoms of pain, itching and irritation were included in the study. While patients with haemorrhoidal complications like swelling, thrombosis, prolapsed and/or ulcers, patients with any serious systemic illness requiring long term medications, women who are pregnant, attempting to conceive, or nursing an infant were excluded from the study. As per randomization, all patients received either the routine standard of care (Syrup Liquid paraffin 30 ml HS oral with lignocaine gel for local application for 4 weeks, along with high fibre diet with plenty of oral fluids and Sitz bath twice a day in both the groups) or Pilex Forte tablet along with Pilex Ointment for a period of 4 weeks at a recommended dose of two tablets twice daily and twice daily local application.Clinical Safety and efficacy assessments were carried out at entry, at the end of 1st week, 2nd week and at the end of 4th week. Laboratory assessments (Haemoglobin, Total Leukocyte Count, Differential Leukocyte Count, Random Blood Sugar, Serum Creatinine and Serum Glutamic Pyruvic Transaminase) were carried out only at screening and at the end of the study. Grading of symptoms were carried out based on VAS scale from 0-10 for pain (0= no pain, 1-3= mild pain, 4-6= moderate, 7-10= Severe pain), constipation/Painful defecation. For bleeding, itching, burning sensation. scoring was based on 5-point Likert scale (0-Nil, 1- Mild, 2- Moderate, 3- Severe and 4- Very severe). Adverse events either reported or observed during the study period were recorded in the case report form. Figure 1 represents the consort study flow.

All patients who completed the study were considered for statistical analysis. Friedman test Followed by Dunnett's multiple comparisons test was used for within group comparisons, Mann Whitney test was used to compare the difference between participants receiving Pilex

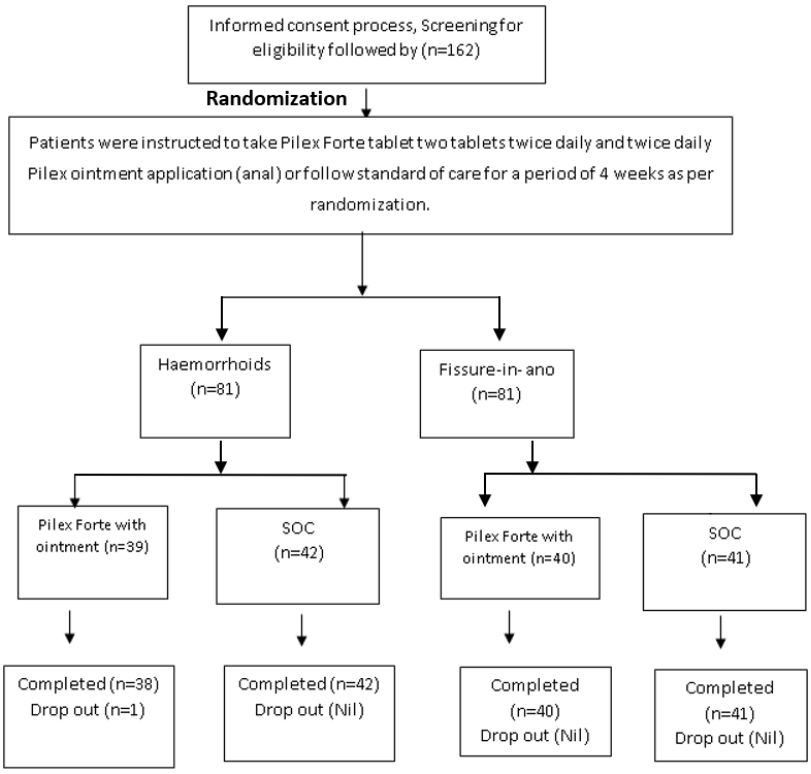

Fig. 1. Consort Study Flow Diagram

Forte and Ointment vs. Standard of care. These tests were performed with a significance level of 0.05 (two-sided). Descriptive statistics was provided for all parameters, Values were represented in Mean ± SD, number of patients and percentages. All statistical analyses were carried out using GraphPad prism software Version 6.07 for Windows, GraphPad Software, San Diego, California, USA.

Results

Total 162 patients were enrolled into the study. 79 patients received Pilex Forte tablets with Pilex Ointment local application and 83 patients received Standard of Care treatment for 4 weeks, 1 patient was not considered for analysis due to lost to follow up and rest all patients completed the study and were considered for statistical evaluation. Table 1 elicits the demographic data of the study.

In haemorrhoid group, 39 patients who received Pilex Forte tablet along with local application of Pilex Ointment were with the mean age of 37.15±9.72 and 42 patients who received Standard of Care treatment were with the mean age of 36.48±9.25.

In fissure-in ano group, 40 patients who received Pilex Forte Tablet along with local application of Pilex Ointment were with the mean age of 37.10±10.07 and 41 patients who received Standard of Care treatment were with the mean age of 38.39±8.78.

From overall 162 patients randomized, 81 patients were included for the assessment of Haemorrhoids (39 patients received Pilex Forte tablets with local application of Pilex Ointment and 42 patients received Standard of Care treatment) and 81 patients were included for the assessment of Fissure-in-ano, out of which 40 patients received Pilex Forte tablet with local application of Pilex Ointment and 41 patients received standard of care treatment alone.

Table 1. Demographics data of patients

	Haemorrhoids group		Fissure-in-ano group	
	Pilex Forte	Standard of Care	Pilex Forte	Standard of Care
Number of patients	39	42	40	41
Age in years	37.15	36.48	37.1	38.39
	9.72	9.25	10.07	8.78
Gender				
Male	20	27	25	24
Female	19	15	15	17

Clinical Assessment of Pilex Forte Tablet with local application of Pilex Ointment in Haemorrhoid Group

Effect on Pain

Figure 2 elicits the effect of Pilex Forte tablets along with local application of Pilex Ointment on pain in haemorrhoids group.

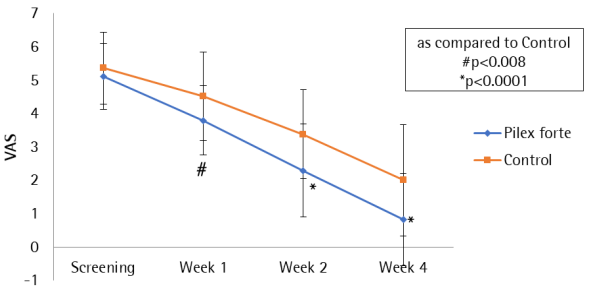

Fig. 2. Effect of Pilex Forte tablets along with local application of Pilex Ointment on pain in haemorrhoids group

Table 2. Clinical Assessment of Pilex Forte tablets with local application of Pilex Ointment in Haemorrhoids Group [Mean±SD]

Parameters	Visits	Pilex Forte	Standard of care
Pain	**Screening**	5.11±0.98	5.36±1.08
	Week 1	3.79±1.04	4.52±1.33
		b:p<0.0048	b:p<0.0264
		c:p<0.0088	
	Week 2	2.29±1.39	3.38±1.34
		b:p<0.0001	b:p<0.0001
		c:p<0.0001	
	Week 4	0.82±1.39	2±1.67
		b:p<0.0001	b:p<0.0001
		c:p<0.0001	
Constipation/ painful defecation	Screening	5.71±1.18	5.86±1.24
	Week 1	4.24±1.17	4.95±1.29
		b:p<0.0056	b:p<0.0337
		c:p<0.0177	
	Week 2	2.66±1.19	3.69±1.46
		b:p<0.0001	b:p<0.0001
		c:p<0.0007	
	Week 4	1.11±1.56	2.38±1.64
		b:p<0.0001	b:p<0.0001
		c:p<0.0001	

Within the group analysis: Statistical test: Friedman test Followed by Dunnett's multiple comparisons test. Significance level was fixed at 0.05; b: as compared to Screening. **Between the group analysis:** Statistical test: Mann Whitney test; c: as compared to Standard of care. Two sided p value; Software: GraphPad Prism 6.07

The mean value of Pain at screening was 5.11±0.98 in Pilex Forte group, it was reduced to 3.79±1.04 at the end of week 1 with a significance of p<0.0048 as compared to screening and p<0.0088 as compared to standard of care which further reduced to 2.29±1.39 at the end of the week 2 with a significance of p<0.0001 as compared to screening and p<0.0001 as compared to standard of care and it reduced to 0.82±1.39 at the end of the week 4 with a significance of p<0.0001 as compared to screening and p<0.0001 as compared to standard of care. In Standard of Care group, the mean value of Pain at screening was 5.36±1.08, it was reduced to 4.52±1.33 at the end of week 1 with a significance of

p<0.0264 as compared to screening, which further reduced to 3.38±1.34 at the end of the week 2 with a significance of p<0.0001 as compared to screening. It was further reduced to 2±1.67 at the end of the week 4 with a significance of p<0.0001 as compared to screening (Table 2).

Effect on constipation/painful defecation

Figure 3 elicits the effect of Pilex Forte tablets along with local application of Pilex Ointment on constipation/ painful defecation in haemorrhoids group.

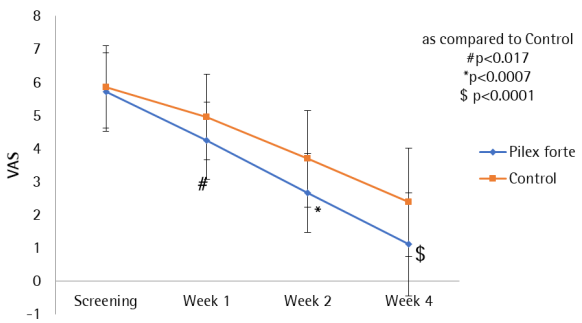

Fig. 3. Effect of Pilex Forte tablets along with local application of Pilex Ointment on constipation/painful defecation in haemorrhoids group

The mean value of constipation/ painful defecation at screening was 5.71±1.18 in Pilex Forte group, it was reduced to 4.24±1.17 at the end of week 1 with a significance of p<0.0056 as compared to screening and p<0.0177 as compared to standard of care which further reduced to 2.66±1.19 at the end of the week 2 with a significance of p<0.0001 as compared to screening and p<0.0007 as compared to standard of care and it reduced to 1.11±1.56 at the end of the week 4 with a significance of p<0.0001 as compared to screening and p<0.0001 as compared to standard of care. In Standard of Care group, the mean value of constipation/ painful defecation at screening was 5.86±1.24, it was reduced to 4.95±1.29 at the end of week 1 with a significance of p<0.0337 as compared to screening, which further reduced to 3.69±1.46 at the end of the week 2 with a significance of p<0.0001 as compared to screening and it was reduced to 2.38±1.64 at the end of the week 4 with a significance of p<0.0001 as compared to screening.

Effect on Itching

Table 2a and Table 2b elicits the effect of Pilex Forte tablets along with local application of Pilex Ointment on the symptoms like itching, bleeding, burning sensation in haemorrhoids group.

In Pilex Forte group, 9 patients presented severe itching, 21 showed moderate itching and 8 patients showed mild itching at screening; however after treatment, 1 patient improved to moderate itching, 1 patient improved to mild itching and 36 patients showed no symptoms of itching at the end of week 4.

In standard of care group, 2 patients had very severe itching, 9 patients presented severe itching, 27 patients showed moderate itching and 4 patients showed mild itching at screening, while after treatment 1 patient showed severe itching, 1 patient showed moderate itching, 9 patients showed mild itching and 31 patients showed no symptoms of itching at end of week 4.

Effect on Bleeding

In Pilex Forte group, 14 patients presented very severe bleeding/streaks, 22 showed severe, 2 showed moderate bleeding/streaks at screening, while after treatment, 1 patient showed very severe bleeding/streaks, 11 patients showed mild bleeding/streaks and 26 patients showed no symptoms of bleeding/streaks at end of week 4.

Table 2a. Clinical assessment of Pilex Forte tablets with local application of Pilex Ointment in haemorrhoids group Pilex Forte group (n=38)

Parameters	Scale	Screening	Week 1	Week 2	Week 4
Itching	Nil	0 (0)	4 (10.53)	25 (65.79)	36 (94.74)
	Mild	8 (21.05)	18 (47.37)	10 (26.32)	1 (2.63)
	Moderate	21 (55.26)	14 (36.84)	2 (5.26)	1 (2.63)
	Severe	9 (23.68)	2 (5.26)	1 (2.63)	0 (0)
	Very severe	0 (0)	0 (0)	0 (0)	0 (0)
Bleeding/ Streaks	Nil	0 (0)	0 (0)	7 (18.42)	26 (68.42)
	Mild	0 (0)	5 (13.16)	15 (39.47)	11 (28.95)
	Moderate	2 (5.26)	13 (34.21)	14 (36.84)	0 (0)
	Severe	22 (57.89)	17 (44.74)	1 (2.63)	0 (0)
	Very severe	14 (36.84)	3 (7.89)	1 (2.63)	1 (2.63)
Burning sensation	Nil	0 (0)	2 (5.26)	19 (50)	36 (94.74)
	Mild	3 (7.89)	16 (42.11)	14 (36.84)	1 (2.63)
	Moderate	26 (68.42)	17 (44.74)	4 (10.53)	1 (2.63)
	Severe	9 (23.68)	3 (7.89)	1 (2.63)	0 (0)
	Very severe	0 (0)	0 (0)	0 (0)	0 (0)

Scale: 0- Nil, 1- Mild, 2- Moderate, 3- Severe, 4- Very severe.

Table 2b. Clinical assessment of Pilex Forte tablets with local application of Pilex Ointment in haemorrhoids group standard of care group (n=42) [N (%)]

Parameters	Scale	Screening	Week 1	Week 2	Week 4
Itching	Nil	0 (0)	1 (2.38)	13 (30.95)	31 (73.81)
	Mild	4 (9.52)	9 (21.43)	24 (57.14)	9 (21.43)
	Moderate	27 (64.29)	29 (69.05)	3 (7.14)	1 (2.38)
	Severe	9 (21.43)	2 (4.76)	1 (2.38)	1 (2.38)
	Very severe	2 (4.76)	1 (2.38)	1 (2.38)	0 (0)
Bleeding/ Streaks	Nil	0 (0)	0 (0)	1 (2.38)	10 (23.81)
	Mild	0 (0)	2 (4.76)	8 (19.05)	16 (38.1)
	Moderate	3 (7.14)	12 (28.57)	16 (38.1)	9 (21.43)
	Severe	16 (38.1)	12 (28.57)	16 (38.1)	7 (16.67)
	Very severe	23 (54.76)	16 (38.1)	1 (2.38)	0 (0)
Burning sensation	Nil	0 (0)	1 (2.38)	8 (19.05)	29 (69.05)
	Mild	4 (9.52)	9 (21.43)	20 (47.62)	6 (14.29)
	Moderate	26 (61.9)	27 (64.29)	13 (30.95)	7 (16.67)
	Severe	10 (23.81)	4 (9.52)	0 (0)	0 (0)
	Very severe	2 (4.76)	1 (2.38)	1 (2.38)	0 (0)

Scale: 0- Nil, 1- Mild, 2- Moderate, 3- Severe, 4- Very severe.

In standard of care group, 23 patients presented very severe bleeding/streaks, 16 patients showed severe and 3 patients showed moderate bleeding/streaks at screening, while after treatment, 7 patients showed severe bleeding/streaks, 9 patients showed moderate bleeding/streaks, 16 patients showed mild bleeding/streaks and 10 patients showed no symptoms of bleeding/streaks at end of week 4.

Table 3. Clinical assessment of Pilex Forte tablets with local application of Pilex Ointment in fissure-in-ano group [Mean±SD]

Parameters	Visits	Pilex Forte	Standard of care
Pain	Screening	7.15±0.89	6.9±0.97
	Week 1	5.03±1.03	5.71±1.03
		b:p<0.0014	b:p<0.004
		c:p<0.0015	
	Week 2	2.9±1.08	4.15±1.22
		b:p<0.0001	b:p<0.0001
		c:p<0.0001	
	Week 4	0.9±1.24	2.76±1.59
		b:p<0.0001	b:p<0.0001
		c:p<0.0001	
Constipation/ painful defecation	Screening	7.2±0.99	6.83±1.14
	Week 1	4.83±1.08	5.71±1.06
		b:p<0.0016	b:p<0.0062
		c:p<0.0004	
	Week 2	2.78±1.05	4.22±1.28
		b:p<0.0001	b:p<0.0001
		c:p<0.0001	
	Week 4	0.88±1.09	2.73±1.58
		b:p<0.0001	b:p<0.0001
		c:p<0.0001	

Within the group analysis: Statistical test: Friedman test Followed by Dunnett's multiple comparisons test. Significance level was fixed at 0.05; b: as compared to Screening. **Between the group analysis:** Statistical test: Mann Whitney test; c: as compared to Standard of care. Two-sided p value; Software: GraphPad Prism 6.07

Effect on Burning sensation

In Pilex Forte group; 9 patients presented severe burning sensation, 26 patients showed moderate burning sensation and 3 patients showed mild burning sensation at screening, while after treatment, 1 patient showed moderate burning sensation, 1 patient showed mild burning sensation and 36 patients showed no symptoms of burning sensation at end of week 4.

In standard of care group; 2 patients presented very severe burning sensation, 10 patients showed severe burning sensation and 26 patients showed moderate burning sensation and 4 patient showed mild burning sensation at screening, while after treatment, 7 patients showed moderate burning sensation, 6 patients showed mild burning sensation and 29 patients showed no symptoms of burning sensation at end of week 4.

Clinical assessment of Pilex Forte tablet with local application of Pilex Ointment in fissure-in-ano group (Table 3)

Effect on Pain

Figure 4 elicits the effect of Pilex Forte tablets along with local application of Pilex Ointment on pain in fissure-in-ano Group.

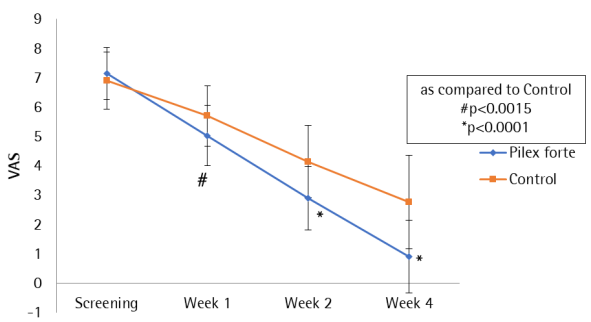

Fig. 4. Effect of Pilex Forte tablets along with local application of Pilex Ointment on pain in fissure-in-ano group

The mean value of pain at screening was 7.15±0.89 in Pilex Forte group, it was reduced to 5.03±1.03 at the end of week 1 with a significance of p<0.0014 as compared to screening and p<0.0015 as compared to standard of care, which further reduced to 2.9±1.08 at the end of the week 2 with a significance of p<0.0001 as compared to screening and p<0.0001 as compared to standard of care and it further reduced to 0.9±1.24 at the end of the week 4 with a significance of p<0.0001 as compared to screening and standard of care.

In Standard of Care group, the mean value of Pain at screening was 6.9±0.97, it was reduced to 5.71±1.03 at the end of week 1 with a significance of p<0.004 as compared to screening which further reduced to 4.15±1.22 at the end of the week 2 with a significance of p<0.0001 as compared to screening and it reduced to 2.76±1.59 at the end of the week 4 with a significance of p<0.0001 as compared to screening.

Effect on constipation/painful defecation

Figure 5 elicits the effect of Pilex Forte tablets along with local application of Pilex Ointment on constipation/ painful defecation in fissure-in-ano group.

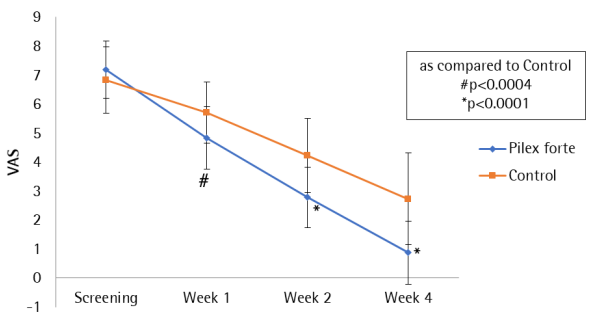

Fig 5. Effect of Pilex Forte tablets along with local application of Pilex Ointment on constipation/painful defecation in fissure-in-ano group.

The mean value of constipation/ painful defecation at screening was 7.2±0.99 in Pilex Forte group, it was reduced to 4.83±1.08 at the end of week 1 with a significance of p<0.0016 as compared to screening and p<0.0004 as compared to standard of care, which further reduced to 2.78±1.05 at the end of the week 2 with a significance of p<0.0001 as compared to screening and standard of care. It further reduced to 0.88±1.09 at the end of the week 4 with a significance of p<0.0001 as compared to screening and standard of care. In Standard of Care group, the mean value of constipation/ painful defecation at screening was 6.83±1.14, it was reduced to 5.71±1.06 at the end of week 1 with a significance of p<0.0062 as compared to screening which further reduced to 4.22±1.28 at the end of the week 2 with a significance of p<0.0001 as compared to screening. It further reduced to 2.73±1.58 at the end of the week 4 with a significance of p<0.0001 as compared to screening.

Table 3a and Table 3b elicits the effect of Pilex Forte tablets along with local application of Pilex Ointment on the symptoms like itching, bleeding, burning sensation in fissure-in-ano group.

Table 3a. Clinical assessment of Pilex Forte tablets with local application of Pilex Ointment in fissure-in-ano group, Pilex Forte Group (n=40) [N (%)]

Parameters	Scale	Screening	Week 1	Week 2	Week 4
Itching	Nil	0 (0)	0 (0)	10 (25)	36 (90)
	Mild	0 (0)	7 (17.5)	24 (60)	2 (5)
	Moderate	3 (7.5)	26 (65)	6 (15)	2 (5)
	Severe	33 (82.5)	6 (15)	0 (0)	0 (0)
	Very severe	4 (10)	1 (2.5)	0 (0)	0 (0)
Bleeding/ Streaks	Nil	0 (0)	0 (0)	13 (32.5)	38 (95)
	Mild	0 (0)	12 (30)	22 (55)	0 (0)
	Moderate	8 (20)	23 (57.5)	4 (10)	1 (2.5)
	Severe	32 (80)	5 (12.5)	1 (2.5)	1 (2.5)
	Very severe	0 (0)	0 (0)	0 (0)	0 (0)
Burning sensation	Nil	0 (0)	0 (0)	15 (37.5)	37 (92.5)
	Mild	0 (0)	13 (32.5)	19 (47.5)	1 (2.5)
	Moderate	5 (12.5)	23 (57.5)	5 (12.5)	1 (2.5)
	Severe	30 (75)	4 (10)	1 (2.5)	1 (2.5)
	Very severe	5 (12.5)	0 (0)	0 (0)	0 (0)

Scale: 0- Nil, 1- Mild, 2- Moderate, 3- Severe, 4- Very severe.

Effect on Itching

In Pilex Forte group; 4 patients presented very severe itching, 33 patients showed severe itching and 3 patients showed moderate itching at screening, while after treatment 2 patients showed moderate itching, 2 patients showed mild itching and 36 patients showed no symptoms of itching at end of week 4. In standard of care group; 4 patients had very severe itching, 32 patients presented severe itching and 5 patients showed moderate itching at screening, while after treatment, 2 patients showed severe itching, 10 patients showed moderate itching, 10 patients showed mild itching and 19 patients showed no symptoms of itching at end of week 4.

Table 3b. Clinical assessment of Pilex Forte tablets with local application of Pilex Ointment in fissure-in-ano group, standard of care group (n=41) [N (%)]

Parameters	Scale	Screening	Week 1	Week 2	Week 4
Itching	Nil	0 (0)	0 (0)	3 (7.32)	19 (46.34)
	Mild	0 (0)	3 (7.32)	13 (31.71)	10 (24.39)
	Moderate	5 (12.2)	20 (48.78)	19 (46.34)	10 (24.39)
	Severe	32 (78.05)	17 (41.46)	6 (14.63)	2 (4.88)
	Very severe	4 (9.76)	1 (2.44)	0 (0)	0 (0)
Bleeding/ Streaks	Nil	0 (0)	0 (0)	3 (7.32)	20 (48.78)
	Mild	0 (0)	2 (4.88)	15 (36.59)	10 (24.39)
	Moderate	6 (14.63)	20 (48.78)	17 (41.46)	10 (24.39)
	Severe	35 (85.37)	19 (46.34)	6 (14.63)	1 (2.44)
	Very severe	0 (0)	0 (0)	0 (0)	0 (0)
Burning sensation	Nil	0 (0)	0 (0)	1 (2.44)	16 (39.02)
	Mild	1 (2.44)	1 (2.44)	13 (31.71)	11 (26.83)
	Moderate	5 (12.2)	21 (51.22)	19 (46.34)	10 (24.39)
	Severe	33 (80.49)	18 (43.9)	8 (19.51)	4 (9.76)
	Very severe	2 (4.88)	1 (2.44)	0 (0)	0 (0)

Scale: 0- Nil, 1- Mild, 2- Moderate, 3- Severe, 4- Very severe.

Effect on Bleeding

In Pilex Forte group; 32 patients presented severe bleeding/streaks and 8 patients showed severe bleeding/streaks at screening, while after treatment, 1 patient showed very severe bleeding/streaks, 1 patient showed moderate bleeding/streaks and 38 patients showed no symptoms of bleeding/streaks at end of week 4. In standard of care group; 35 patients presented severe bleeding/streaks and 6 patients showed moderate bleeding/streaks at screening, while after treatment, 1 patient showed severe bleeding/streaks, 10 patients showed moderate bleeding/streaks, 10 patients showed mild bleeding/streaks and 20 patients showed no symptoms of bleeding/streaks at end of week 4.

Effect on Burning sensation

In Pilex Forte group, 5 patients presented very severe burning sensation, 30 patients showed severe and 5 patients showed moderate burning sensation at screening, while after treatment 1 patient showed severe burning sensation, 1 patient showed moderate burning sensation, 1 patient showed mild burning sensation and 37 patients showed no symptoms of burning sensation at end of week 4. In standard of care group; 2 patients presented very severe burning sensation, 33 patients showed severe and 5 patients showed moderate burning sensation and 1 patient showed mild burning sensation at screening, while after treatment, 4 patients showed severe burning sensation, 10 patients showed moderate burning sensation, 11 patients showed mild burning sensation and 16 patients showed no symptoms of burning sensation at end of week 4.

Overall Comparative Assessment of Pilex Forte with Standard of Care

Table 4a and 4b elicits the overall comparative assessment of Pilex Forte tablets along with local application of Pilex Ointment with standard of care alone by patient & by investigator as assessed at the end of the study (graphically represented in figure 6 and 7).

Table 4a. Overall comparative assessment of Pilex Forte tablets with local application of Pilex Ointment in haemorrhoids Group

Scale	Pilex Forte N (%)		Standard of care N (%)	
	By patient	By investigator	By patient	By investigator
Cured	13 (34.21)	13 (34.21)	4 (9.52)	4 (9.52)
Marked improvement	21 (55.26)	21 (55.26)	12 (28.57)	11 (26.19)
Moderate improvement	3 (7.89)	3 (7.89)	13 (30.95)	14 (33.33)
Slight improvement	1 (2.63)	1 (2.63)	11 (26.19)	11 (26.19)
No change	0 (0)	0 (0)	1 (2.38)	1 (2.38)
Symptoms became worse	0 (0)	0 (0)	1 (2.38)	1 (2.38)

Table 4b. Overall comparative assessment of Pilex Forte tablets with local application of Pilex Ointment in fissure-in-ano group

Scale	Pilex Forte N (%)		Standard of care N (%)	
	By patient	By investigator	By patient	By investigator
Cured	16 (40)	20 (50)	1 (2.5)	1 (2.5)
Marked improvement	20 (50)	17 (42.5)	5 (12.5)	6 (15)
Moderate improvement	3 (7.5)	1 (2.5)	22 (55)	21 (52.5)
Slight improvement	1 (2.5)	2 (5)	12 (30)	12 (30)
No change	0 (0)	0 (0)	0 (0)	0 (0)
Symptoms became worse	0 (0)	0 (0)	0 (0)	0 (0)

In Haemorrhoid group; who received Pilex tablets along with local application of Pilex Ointment, 13 (34.32%) patients out of 38 patients were completely symptom free at the end of 4 week as compared to only 04 (9.52%) patients out of 42 patients who received standard of care alone.

In Fissure-in-ano group; who received Pilex tablets along with local application of Pilex Ointment, 16 (40%) patients out of 40 patients were completely symptom free at the end of 4 week as compared to only 01 (2.5%) patients out of 40 patients who received standard of care alone.

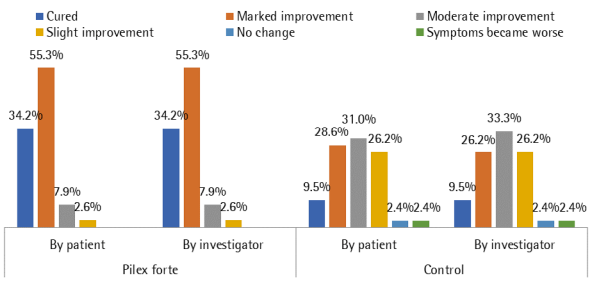

Fig. 6. Overall effect of Pilex Forte tablets with local application of Pilex Ointment in haemorrhoids Group

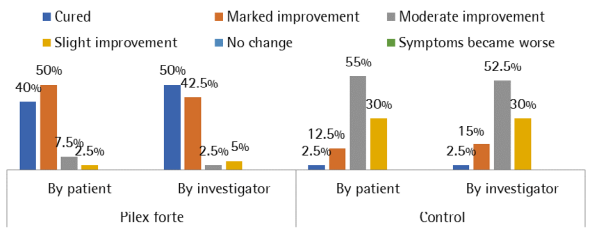

Fig. 7. Overall effect of Pilex Forte tablets with local application of Pilex Ointment in fissure-in-ano group

Discussion

Anorectal disorders are a common reason for visits to both primary care physicians and gastroenterologists/surgeons. These disorders are varied and include benign conditions such as haemorrhoids to more serious conditions such as malignancy; thus, it is important for the clinician to be familiar with these disorders as well and know how to obtain an appropriate history and conduct physical examination. Some of the most common anorectal disorders, including haemorrhoids are anal fissures, fecal incontinence (FI), proctalgia fugax, excessive perineal descent, and pruritus ani.

Pilex Forte tablet and Pilex Ointment for local application is specially formulated for the treatment of various anorectal conditions like Haemorrhoids and fissure–in-ano.

The laxative property of Pilex Forte tablet corrects chronic constipation associated with haemorrhoids. The therapeutic effects of various ingredients in Pilex Ointment are also stated to be anti-inflammatory, laxative and styptic. Pilex Forte tablet contains ingredients such as *Balsamodendron mukul (Purified), Shilajeet (Purified), Melia azadirachta, Berberis aristata, Emblica officinalis, Terminalia chebula, Terminalia belerica, Cassia fistula, Bauhinia variegata, Mesua férrea* with the following pharmacological actions which might possibly be responsible for their synergistic action in bringing about significant clinical improvement in both haemorrhoidal and fissure-in-ano group as appreciated in the present study. The individual herbal actives and its actions with few anecdotal references are detailed as follows,

Balsamodendron mukul (Syn. ***Commiphora mukul***)
The guggulusterone fraction of gum Guggul exhibited anti-inflammatory activity in the models of acute inflammation, which was comparable to approximately one-fifth that of hydrocortisone and equal to phenylbutazone and ibuprofen.[16]

Shilajeet (Purified)
Shilajit (Purified) have been used as anti-inflammatory agents for ages. There are reported results of clinical use of Shilajeet(Purified) in diabetes, fever, anemia, anorexia, spasmodic pain, obesity, abdominal disorders, and skin diseases. It also has cardiotonic activities.[17-18]

Melia azadirachta **(Syn.** ***Azadirachta indica*****)**
The leaf & bark has antimicrobial, antifungal, anthelmintic, insecticidal, antiviral, antipyretic, anti-inflammatory activities. The same is used in inflammatory gum diseases, boils, sores, measles, small pox, and other cutaneous infections.[19-20]

Berberis aristata
The extract has bitter, cholagogue, antidiarrhoeal, stomachic, laxative, antipyretic, antiseptic activities. The same also has anti-inflammatory, hypotensive, and antiamoebic activities.[21] The decoction is used for gastric disorders, piles, and other allied complaints. It gives good relief in senile pruritus. It has laxative activity too that is helpful in managing haemorrhoids.[22]

Emblica officinalis
It has antiviral, antibacterial and antiallergy activities. It is rich in phenols. Aqueous extract showed antibacterial activity against *S. aureus*.[23] Its varied therapeutic activity leads its use in viral hepatitis, premature atherosclerosis, and managing anemia, pimples, fistula *etc*.[24]

Terminalia chebula
It has shown antibacterial, antifungal activity against number of dermatophytes, and improves the immunity to protect from viral growth and infections. Additional antispasmodic and potent wound healing activity by improved rates of contraction and decreased period of ep-

ithelialization were also established. Purgative action of *Terminalia chebula* is also noted. It's widely prescribed in constipation, ulcer, gastroenteritis, cough, haemorrhoids and other skin diseases.[25]

Terminalia belerica

It shows hepatoprotective action against other common hepatotoxic agents.[26] It shows gastroprotective, laxative activity being one of the ingredients of Triphala.[27]

Cassia fistula

In traditional medicines, the same is used in haematemesis, pruritus, leucoderma, and diabetes and other commonly dermatological infestations, inflammatory disorders, and constipation.[28] *C. fistula* pod infusion could be safely utilized as laxative drugs and as a substitute for the official Senna.[29]

Bauhinia variegata

It's widely used in constipation as laxative, antibacterial and blood purifier activities, antioxidant activity and tumours, anti-tumour property and hepatoprotective activity.[31]

Mesua ferrea

Mesua ferrea have potent broad-spectrum antimicrobial actions and prevent secondary microbial infections.[32] It also have astringent and styptic actions that are useful in bleeding piles.[33]

Pilex Ointment and its pharmacology

Pilex Ointment is a herbo-mineral formulation consisting extracts of *Mimosa pudica, Eclipta alba, Vitex negundo, Calendula officinalis, Mimosa pudica, Eclipta alba, Vitex negundo, Calendula officinalis, Cinnamomum camphora, Tankana, Yashada bhasma* which have anti-inflammatory, analgesic, wound healing, membrane stabilizing, anti-microbial and antioxidant actions. Synergistic actions of these potent herbs might possibly bring about localized reduction of varicosities of venous plexus in common ano-rectal conditions like haemorrhoids and fissure-in-ano. The individual herbal actives and its actions with few anecdotal references are detailed as follows,

Mimosa pudica

Mimosa pudica has astringent and styptic actions that are useful in bleeding piles.[34]

Eclipta alba

Eclipta alba has anti-inflammatory and analgesic activities.[35] These actions are mediated via prostaglandin synthesis inhibition and cell membrane stabilizing activities.

Vitex nigundo

This has potent broad-spectrum antimicrobial action; and prevents secondary microbial infections.[36] This also has potent analgesic[37], significant and directly dose-dependent analgesic activity.[38] *Vitex negundo* has strong antihistaminic activity, which helps to control associated itching and also membrane stabilizing and antioxidant activities.[38] Additionally, anti-inflammatory activity due to presence of triterpenoids and anti-histamine activity were also evident.[39]

Calendula officinalis

Calendula officinalis has anti-inflammatory and analgesic activities. *Calendula officinalis* accelerates the wound healing by the epithelial regeneration.[40] Pilex Ointment improves the network of basal membrane collagenous substance, which leads to renormalization of the capillary membranes resistance and subsequently, it induces reduction in the hemorrhoidal mass of the dilated veins of hemorrhoidal plexus.[40] *Calendula officinalis* have potent free radical scavenging activities.[41]

Cinnamomum camphora

Cinnamomum camphora has analgesic activity. It relieves or soothes pain by reducing the sensitivity of the brain or nervous system to pain.[42] It accelerates wound-healing by epithelial regeneration and have potent broad-spectrum antimicrobial actions and prevent secondary microbial infections.[43] It also has potent free radical scavenging activities.[44]

Tankana

Tankana has potent broad-spectrum antimicrobial actions and prevent secondary microbial infections.[45]

Yashada Bhasma

Yashada bhasma, have potent anti-inflammatory and analgesic actions. It accelerates wound-healing by epithelial regeneration.[46]

Conclusion

Based on the results of the present clinical study, it was clearly evidential that Pilex Forte tablets along with the local application of Pilex Ointment showed significant clinical improvement with reduced clinical signs and symptoms in both Haemorrhoids and fissure-in-ano as compared to Standard of care alone. No serious or non-serious adverse events were reported or observed throughout the study period. It can be concluded that Pilex Forte tablet along with the local application of Pilex Ointment is safe and effective in the treatment of anorectal conditions like haemorrhoids and fissure-in-ano.

References

1. Henderson PK, Cash BD. Common anorectal conditions: evaluation and treatment. *Curr Gastroenterol Rep*. 2014;16(10):408.

2. Di Palma JA. Introducing comprehensive non-surgical anorectal care to the gastroenterology fellowship training curriculum: University of South Alabama experience. *Pract Gastroenterol*. 2011;35:31-36.
3. Ganz R. The evaluation and treatment of haemorrhoids: a guide for the gastroenterologist. *Clin Gastroenterol and Hepatol*. 2013;11:593-603.
4. Appalaneni V, Fanelli R, Sharaf R, American Society for Gastrointestinal Endoscopy, Standards of Practice Committee, et al. The role of endoscopy in patients with anorectal disorders. *Gastrointest Endosc*. 2010;72:1117-1123.
5. Riss S, Weiser FA, Schwameis K, et al. The prevalence of haemorrhoids in adults. *Int J Colorectal Dis*. 2012;27(2):215-220.
6. Hall J. Modern management of hemorrhoidal disease. *Gastroenterol Clin N Am*. 2013;42:759-772.
7. Jacobs D. Clinical practice. Haemorrhoids. *N Engl J Med*. 2014;371(10):944-951.
8. Mounsey AL, Halladay J, Sadiq TS. Haemorrhoids. *Am Fam Physician*. 2011;84(2):204-210
9. Alonso-Coello P, Guyatt G, Heels-Ansdell D, et al. Laxatives for the treatment of haemorrhoids. *Cochrane Database Syst Rev*. 2005;4:CD004649.
10. Shafik A. Role of warm-water bath in anorectal conditions. The "thermosphincteric reflex." *J Clin Gastroenterol*. 1993;16(4):304-308.
11. Lohsiriwat V. Haemorrhoids: from basic pathophysiology to clinical management. *World J Gastroenterol*. 2012;18(17):2009-2017.
12. Lacy BE, Weiser K. Common anorectal disorders: diagnosis and treatment. *Curr Gastroenterol Rep*. 2009;11: 413-419.
13. Lysy J, Sistiery-Ittah M, Israelit Y, et al. Topical capsaicin--a novel and effective treatment for idiopathic intractable pruritus ani: a randomized, placebo-controlled, crossover study. *Gut*. 2003;52:1322-1326.
14. Jensen SL. Treatment of first episodes of acute anal fissure: prospective randomized study of lignocaine Ointment versus hydrocortisone Ointment or warm sitz baths plus bran. *Br Med J*. 1986;292:1167-1169.
15. Dhawan S, Chopra S. Nonsurgical approaches for the treatment of anal fissures. *Am J Gastroenterol*. 2007;102:1312-1321.
16. Khare CP. *Encylopedia of Indian Medicine*. Germany: Springer; 2007:159-160.
17. Ayurvedic Formulary of India. Part-II. First English Edition. Ministry of H & FW, Dept. of ISM & H. Govt of India. New Delhi. 2000:373-374
18. Mishra S. Ayurvediya Rasashastra. Chaukhamba Orientalia. Varanasi. 7th edition. 1997: 399.
19. Khare CP. Indian Medicine: An illustrated dictionary. New Delhi: Springer; 2007:75.
20. Kausik Biswas, Ishita Chattopadhyay, Ranajit K. Banerjee, Uday Bandyopadhyay. Biological activities and medicinal properties of neem *(Azadirachta indic*a). *Current Science*. 2002;82(11):June.
21. Khare CP. *Indian Medicine: An illustrated dictionary*. New Delhi: Springer;2007:88-89.
22. Asolkar LV. *Second supplement to glossary of Indian Medicinal Plants with active principles*. New Delhi: CSIR. Govt. of India; 1992:120.
23. Rathish Nair, Sumitra V. Chanda. Antibacterial Activities of Some Medicinal Plants of the Western Region of India. *Turk J Biol*. 2007;31:231-236.
24. Asolkar LV, et al. *Second supplement to glossary of Indian Medicinal Plants with active principles*. New Delhi: CSIR. Govt. of India; 1992:291-292.
25. Chattopadhyay RR, Bhattacharyya SK. *Terminalia chebula*: An update. *Pharmacognosy Reviews*. 2007;1(1):151-156.
26. Khare CP. *Indian Medicine: An illustrated dictionary*. New Delhi: Springer; 2007:652653.
27. Yoganarasimhan SN. *Medicinal Plants of India - Tamil Nadu, Bangalore*. 1st Edition, 2000:540.
28. Asolkar LV. Second supplement to glossary of Indian Medicinal Plants with active principles. New Delhi: CSIR. Govt. of India; 1992:177-178.
29. Akanmu MA, Iwalewa EO, Elujoba AA, Adelusola KA. Toxicity Potentials of *Cassia Fistula* Fruits as Laxative with Reference to Senna. *African J Biomed Res*. 2004;7:23-26.
30. Asolkar LV, et al. *Second supplement to glossary of Indian Medicinal Plants with active principles*. New Delhi: CSIR. Govt. of India; 1992:113-114.
31. Mali RG, Mahajan SG, Mehta AA. Rakta Kanchan (*Bauhinia variegata*): Chemistry, Traditional and Medicinal uses- a review. *Pharmacognosy Reviews*. 2007;1(2):Jul-Dec.
32. Mazumder R, Dastidar SG, Basu SP, Mazumder A, Singh SK. Antibacterial potentiality of *Mesua ferrea* Linn. flowers. *Phytother Res*. 2004;18(10):824-826.
33. Chopra RN, Nayar SL, Chopra LC. *Mesua ferrea*. *Glossary of Indian Medicinal Plants*. National Institute of Science Communication, New Delhi. 4th Reprint, 1996:166.
34. Chopra RN, Nayar SL, Chopra LC. *Mimosa pudica*. Glossary of Indian Medicinal Plants. National Institute of Science Communication, New Delhi. 4th Re-print, 1996:167.
35. Leal LK, Ferreira AA, Bezerra GA, Matos FJ, Viana GS. Antinociceptive, anti-inflammatory and bronchodilator activities of Brazilian medicinal plants containing coumarin: A comparative study. *J Ethnopharmacol*. 2000;70(2):151-159.
36. Perumal Samy R, Ignacimuthu S, Sen A. Screening of 34 Indian medicinal plants for antibacterial properties. *J Ethnopharmacol*. 1998;62(2):173-182.
37. Jagetia GC, Baliga MS. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants *in vitro*: a preliminary study. *J Med Food*. 2004;7(3):343-348.
38. Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J Ethnopharmacol* 2003;87(2-3):199-206.
39. Chawla AS, Sharma AK, Handa SS, Dhar KL. Chemical investigation and anti-inflammatory activity of *Vitex negundo* seeds. *J Nat Prod*. 1992;55(2):163-167.

40. Lavagna SM, Secci D, Chimenti P, Bonsignore L, Ottaviani A, Bizzarri B. Efficacy of Hypericum and Calendula oils in the epithelial reconstruction of surgical wounds in childbirth with caesarean section. *Farmaco.* 2001;56(5-7):451-453.
41. Cordova CA, Siqueira IR, Netto CA, et al. Protective properties of butanolic extract of the *Calendula officinalis* L. (marigold) against lipid peroxidation of rat liver microsomes and action as free radical scavenger. *Redox Rep.* 2002;7(2):95-102.
42. Chopra RN, Nayar SL, Chopra LC. *Cinnamomum camphora*. Glossary of Indian Medicinal Plants. National Institute of Science Communication, New Delhi. 4th Re-print, 1996:65.
43. Asolkar LV, Kakkar KK, Chakre OJ. *Cinnamomum camphora*. Glossary of Indian Medicinal plants with Active principles. Publications & Information Directorate (CSIR), New Delhi. 1992; Second supplement, Part –I (A-K), (1965-1981):203.
44. Lee HJ, Hyun EA, Yoon WJ, et al. *In vitro* anti-inflammatory and anti-oxidative effects of *Cinnamomum camphora* extracts. *J Ethnopharmacol.* 2006;103(2):208-216.
45. Siddhinandan Mishra. Tankana. Ayurvediya Rasashastra. Chaukhambha Orientalia, Varanasi. 7th edition. 1997:699-702.
46. Sri Sadananda Sharma. Rasatarangini. Yashada bhasma. Motilal Vanarasi Das, Delhi. 11th edition., 1994:482-483.

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ORIGINAL PAPER

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The relationship between mindful eating, body mass index and physical activity in nursing students – a cross-sectional study

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ABSTRACT

Introduction. One of the reasons of obesity in university students might be mindful eating.

Aim. This study was performed to evaluate the relationship between mindful eating, body mass index (BMI) and physical activity in nursing students.

Material and methods. This cross-sectional study was conducted with 718 nursing students in a university in Turkey. "Personal Information Form" and "Mindful Eating Questionnaire (MEQ)" were used as data collection tools.

Results. Statistically significant differences were found between some mean subscale and total scores of MEQ based on sex of the students, their state of exercising regularly, number of their meals, their state of having snacks, the type of bread they often consumed and the frequency of eating fast foods ($p<0.05$). A significant relationship was found between age and BMI of the students and their mean subscale and total scores of MEQ ($p<0.05$).

Conclusion. The older the nursing students grew, the higher their level of mindful eating became. Sociodemographic characteristics such as sex and presence of a person with obesity within the family affected subscales of their mindful eating. Mindful eating decreased as body mass index increased; and besides, mindful eating increased as physical activity increased.

Keywords. appetite, mindful eating, obesity

Introduction

Obesity is one of the chronic diseases that are qualified among endemic diseases affecting every age group today. Obesity prevalence especially among university students has been reported to be high due to several reasons.[1,2] In the prevalence study by Peltzer et al. which was performed to determine obesity level among university students in 22 different countries, 18.9 % of the

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male students were overweight, 5.8 % of them were individuals with obesity and 14.1 % of the female students were overweight and 5.2 % of them were individuals with obesity.[2] Basu et al. reported in the study conducted with 278 university students that 28% of them were individuals with obesity and 20 % were overweight.[1] Obesity is the etiological factor in the origin of many diseases such as cardiovascular diseases, Type II diabetes, metabolic syndrome and cancer.[3] Therefore, interventions have great importance in the prevention of university students from obesity.

Most studies conducted to prevent the obesity among university students were generally performed to identify wrong dietary behaviors, promote healthy dietary behaviors and physical activity.[4,5] However, only interventions may not be enough for obesity since young adulthood period is now evaluated in the scope of adolescence period and these children should cope with the psychological and hormonal problems of adolescence period. In addition, having a dormitory life away from the family may negatively affect healthy dietary behaviors of the students materially and morally.[4]

The significant point of healthy nutrition is the generation of nutritional awareness. Nutritional awareness brings along mindful eating. Mindful eating is defined as "paying attention to an eating experience with all of our senses (seeing, tasting, hearing, smelling, feeling); witnessing the emotional and physical responses that take place before, during and after the eating experience. Mindful eating is a type of eating that requires focusing on the food to be consumed by noticing what, how and why the individual eats, by having a hunger-satiety awareness, by realizing his/her eating behaviours and without being affected from environmental factors.[6,7] Fundamentally, mindful eating involves eating slowly and without distraction, listening to physical hunger cues and eating only until you're full, distinguishing between real hunger and non-hunger triggers for eating, engaging your senses by noticing colors/smells/sounds/textures/flavors, learning to cope with guilt and anxiety about food, eating to maintain overall health and well-being, noticing the effects food has on your feelings and figure, and appreciating the food. These things allow you to replace automatic thoughts and reactions with more conscious, healthier responses. Mindful eating is a technique that helps you gain control over your eating habits. Mindful eating has been shown to promote weight loss, reduce overeating, and help person feel better.[8,9]

Aim

This study was performed to evaluate the association between mindful eating, body mass index (BMI) and physical activity in university students. In line with this purpose, this research will contribute to the literature on the following issues: (1) Estimation of the relationship between mindful eating and the physical activity can contributes to the decrease in obesity. (2) If the nurses are knowledgeable enough about mindful eating, they could advise people about the mindful eating both in clinics and social environments. The questions of this study areas follows:

1. Are there differences in mindful eating levels based on sex, family history of obesity, regular exercise, number of meals, snacks, balanced diet, type of bread consumed, frequency of fast food consumption?
2. Are there correlations between mindful eating scores, age and BMI?

Material and methods

Setting and sample

This cross-sectional study was carried out in a university in the Western Black Sea Region of Turkey. The study was conducted with nursing students due to their roles of health education and public awareness.The World Health Organization (WHO), on the other hand, defines 10-19 years as adolescence, 15-24 years as youth, and 10-24 years as young people, but considers these age groups within adolescent health.[10] The Healthy People Report has stated that the adolescence period extends up to the age of 25.[11] Our study was carried out on nursing students between the ages of 18-25 in adolescence. The universe of the study was composed of 944 nursing students in 2017-2018 academic years. The study was performed on whole universe without choosing a sample. The sample of the study included 718 nursing students who agreed to participate in the study during assigned dates (76.0% of the universe was reached). The inclusion criteria of the study are being a student in the departments of health, and a volunteer to participate in the study. The exclusion criteria of the study are not being a student in the departments of health and a volunteer to participate in the study.

In this study, 70.2% students were females, 29.8% were males, 55.4% were within the age of 21-25, 49.3% were within the weight of 60-90 kg, 77.9% were within the height of 160-180 cm, 3.5% had a problem with obesity, 15% were overweight, 72% were normal and 9.5% were underweight.

Data collection tools

The data were collected by "Personal Information Form" and "Mindful Eating Questionnaire (MEQ-30)".

Personal Information Form: This was a questionnaire form including open and close-ended questions prepared in accordance with the literature and expert opinions to identify the factors affecting sociodemographic characteristics and obesity.[6,7]

Mindful Eating Questionnaire (MEQ-30): Five items were taken from Mindful Eating Questionnaire (MEQ-28), and the remaining items were adapted from the same scale again; and the new scale was generated

with a total of 30 questions. When Likert-type scales are used in the studies, the number of options is generally five. For that reason, a 5-Likert type scale was used in the new version (1: never, 2: rarely, 3: sometimes, 4: often, 5: always). The subscales of the scale was classified into seven factors which were "Disinhibition", "Emotional Eating", "Eating Control", "Distraction", "Eating Discipline", "Awareness and Interference".[9]

Data collection

The study was conducted by the researchers between 03.01.2018-05.31.2018. The students were informed about the aim of the study in the classes at the arranged hours, and they were asked to participate in the study. Participation was on voluntary basis. Questionnaires were handed out to the students and they were asked to fill it out after they were informed briefly during the study.

Ethical considerations

An ethics committee approval and institutional authorization were obtained from Zonguldak Bülent Ecevit University Human Research Ethics Committee to conduct the study. The required written authorization was also obtained to use "Mindful eating questionnaire (MEQ-30).

Data assessment

Statistical Package for the Social Sciences 22.0 was used for statistical assessment. The normality of the data was assessed by Kolmogorow Smirnov test. In descriptive statistics, numerical data were expressed by mean ± standard deviation (minimum-maximum) and categorical data were expressed as numbers and percentages. Since parametric test assumptions could not be provided for numerical variables, Mann-Whitney U and Kruskal Wallis tests were used to compare both groups. Tukey test was used to determine where the difference originates in multiple comparisons. Correlation was assessed by Spearmen rho's test. The results were analyzed within a confidence interval of 95% and $p<0.05$ was considered as statistically significant.

Results

In this study, 64.5% had an irregular eating habit, 53.5% were eating a fixed menu at lunch, 58.8% were eating a fixed menu at dinner, 34.8% were eating candies, chocolates, etc. from canteen and 69.8% were often eating white bread. 23% students were smoking, 15.3% were consuming alcoholic drinks, 86.2% were eating fast-food and 64,8% were consuming sugar. Moreover,

Table 1. Comparison of mean scores from subscales of mindful eating questionnaire based on descriptive characteristics and physical activities (n=718)

Mindful eating questionnaire subscales	Characteristics					
	Sex		Familial history of obesity		Regular exercise	
	Female	Male	Yes	No	Yes	No
Disinhibition Mean±SD (min-max)	15.66 ± 3.86 (5-25)	15.53 ±11.12 (8-25)	15.29 ± 3.78 (5-25)	15.68 ± 3.75 (8-25)	16.19 ± 4.39 (5-25)	15.42 ± 3.48 (5- 25)
	U: -0.532; p: 0.594		U: -0.960; p: 0.908		**U: 2.416; p: 0.001**	
Emotionaleating Mean±SD (min-max)	15.00 ± 4.55 (5-25)	15.90 ± 11.12 (5-25)	15.50 ± 4.43 (5-25)	15.23 ± 4.58 (5-25)	15.8 ± 4.81 (5-25)	15.07 ± 4.45 (5-25)
	U: -2.101; p: 0.036		U: 0.572; p: 0.692		U: 1.982; p: 0.110	
Eatingcontrol Mean±SD (min-max)	13.87 ± 3.13 (4-20)	13.13 ± 11.12 (6-20)	12.90 ± 3.01 (4-20)	13.78 ± 3.06 (6-20)	13.50 ± 3.14 (6-20)	13.70 ± 3.04 (4-20)
	U: -3.040; p: 0.002		U: -2.705; p: 0.859		U: -0.786; p: 0.433	
Distraction Mean±SD (min-max)	15.18 ± 2.31 (8-24)	15.34 ± 11.12 (8-24)	15.16 ± 2.32 (8-24)	15.24 ± 2.33 (8-24)	15.17 ± 2.47 (9-23)	15.25 ± 2.27 (8-24)
	U: -0.744; p:0.457		U: -0.333; p: 0.713		U: -0.408; p: 0.100	
Eatingdiscipline Mean±SD (min-max)	11.34 ± 2.95 (4-20)	11.53 ± 11.12 (4-19)	11.37±3.30 (4-20)	11.40 ± 2.90 (4-19)	12.00 ± 3.39 (4-20)	11.18 ± 2.76 (4-19)
	U: -0.752; p: 0.452		U:-0.102; p: 0.060		**U:3.276; p: 0.003**	
Awareness Mean±SD (min-max)	14.73 ± 2.56 (8-22)	14.03 ± 11.12 (8-20)	14.52 ± 2.20 (8-22)	14.52 ± 2.61 (8-20)	14.86 ± 2.61 (9-22)	6.68 ± 1.76 (2-10)
	U: -3.301; p: 0.001		U: 0.010; p: 0.034		U: 2.141; p: 0.964	
Interference Mean±SD (min-max)	6.73 ± 11.12 (2-10)	6.62 ± 11.12 (2-10)	6.56 ± 1.94 (2-10)	6.72 ± 1.84 (2-10)	6.75 ± 2.09 (2-10)	6.68 ± 1.76 (2-10)
	U: -0.752; p: 0.452		U: -0.808; p: 0.386		**U: 0.459; p: 0.001**	
Total Mean±SD (min-max)	92.54 ± 11.12 (57-123)	92.10 ± 10.39 (66-131)	91.34 ± 10.01 (60-117)	92.59 ± 11.04 (57-131)	94.33 ± 12.02 (60-131)	91.73 ± 10.40 (57-123)
	U: -0.542; p: 0.588		U: 0.808; p: 0.386		**U: 2.826; p: 0.011**	

U:Mann Whithey U test.

73,7% of the students were not exercising regularly and 14.5% had a familial history of obesity.

Table 2. Correlation between mindful eating scores, age and body mass index in students who included in the study (n=718)

Mindful eating questionnaire	Age		Body Mass Index	
	r	*p*	*r*	*p*
Disinhibition	0.075	**0.045**	-0.094	**0.012**
Emotional eating	0.124	**0.001**	-.106	**0.004**
Eating control	0.028	0.452	-0.171	**<0.001**
Distraction	0.068	0.070	0.033	0.378
Eating discipline	0.021	0.569	0.078	**0.037**
Awareness	-.033	0.372	-0.031	0.400
Interference	0.67	0.71	-1.111	**0.003**
Total score	-0.175	**<0.001**	0.074	**0.048**

The lowest mean score was obtained from interference factor (6.70±1.85), eating discipline (11.40±2.96), eating control (13.65±3.06) and awareness (14.52±2.55), respectively. It was seen that the lowest score obtained by the students from MEQ-30 was 57, and the highest score was 131.

Statistically significant differences were found between their scores of emotional eating, eating control, and awareness subscales based on the sex (p<0.05). In this study, awareness of males was found to be higher in emotional eating, eating control and awareness than the females. Statistically significant differences were found between the scores in awareness subscale based on the familial history of obesity (p<0.05). Emotional eating and eating control awareness of the students who had a familial history of obesity were low. Statistically significant differences were found between their subscale scores of disinhibition, eating discipline and interference, and their score of total scale based on their physical activity characteristics (p<0.05). Eating discipline, interference and mindful eating in general were found to be high among the students who were exercising regularly (Table 1).

A positive correlation was found between ages and disinhibition, emotional eating subscales. A negative and significant correlation was found between age and total MEQ-30 score (p<0.05). As the age of the students became older, their emotional eating increased, but not their eating awareness. A negative and significant correlation was found between BMI and disinhibition, emotional eating, eating control; and a positive correlation was found between BMI and eating discipline, total MEQ-30 score (p<0.05). As the BMI of the students increased, eating discipline and eating awareness raised but not their disinhibition, emotional eating, eating control (Table 2).

Significant differences were found between their subscale scores of eating discipline based on the number of their meals and emotional eating according to

Table 3. Comparison of mean scores of students from Mindful Eating Questionnaire based on their eating habits (n=718)

Mindful eating questionnaire subscales	Characteristics						
	Number of meals			Snacks		Balanced diet	
	1	2	3	Yes	No	Yes	No
Disinhibition	16.68 ± 4.60	15.79 ± 3.63	15.44 ± 3.78	15.63 ± 3.79	15.61 ± 3.72	16.35 ± 3.75	15.37 ± 3.73
Mean±SD	(8-25)	(5-25)	(5-25)	(5-25)	(5-25)	(5-25)	(5-25)
(min-max)	KW: 0.345; p: 0.238			U: 0.040; p: 0.343		**U: -3.392; p: 0.001**	
Emotional eating	16.16 ± 4.52	15.30 ±4.68	15.19 ±4.48	15.35 ±4.31	15.19 ± 4.79	16.23 ± 4.45	14.94 ± 4.55
Mean±SD	(9-25)	(5-25)	(5-25)	(5-25)	(5-25)	(5-25)	(5-25)
(min-max)	KW: 0.832; p: 0.713			**U: 0.460; p: 0.030**		**U: -3.253; p: 0.001**	
Eating control	13.32 ± 2.89	13.55 ±2.97	13.74 ±3.14	13.72 ±3.09	13.58 ± 3.04	14.01 ± 3.15	13.53 ± 3.03
Mean±SD	(8-20)	(6-20)	(4-20)	(4-20)	(4-20)	(4-20)	(4-20)
(min-max)	KW: 0.892; p: 0.687			U: 0.586; p: 0.921		U: -1,711; p: 0.087	
Distraction	15.12 ± 2.55	15.05 ±2.39	15.36 ±2.26	15.22 ±2.30	15.23 ± 2.35	15.18 ± 2.13	15.24 ± 2.39
Mean±SD	(12-24)	(8-24)	(8-22)	(9-24)	(8-23)	(10-22)	(8-24)
(min-max)	KW: 0.322; p: 0.124			U: -0.066; p: 0.770		U: -0.232; p: 0.816	
Eating discipline	10.32 ± 3.35	11.15 ± 2.97	11.64±2.91	11.58±2.95	11.22 ± 2.97	12.27 ± 3.16	11.10±2.84
Mean±SD	(4-16)	(4-20)	(4-19)	(4-20)	(4-20)	(4-20)	(4-20)
(min-max)	**KW: 0.752; p: 0.024**			U: 1.640; p: 0.836		**U: -4.761; p: <0.000**	
Awareness	14.04 ± 2.93	14.54 ±2.42	14.54±2.62	14.69±2.66	14.36 ± 2.44	15.05 ± 2.57	14.34±2.53
Mean±SD	(8-18)	(9-22)	(8-22)	(8-22)	(8-22)	(10-22)	(8-22)
(min-max)	KW: 0.432; p: 0.818			U: 1.697; p: 0.093		**U: -2.864; p: 0.004**	
Interference	6.84 ± 1.97	6.75 ± 1.80	6.65 ± 1.89	6.69 ± 1.88	6.70 ± 1.82	7.01 ± 2.03	6.59 ± 1.78
Mean±SD	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)
(min-max)	KW: 0.982; p: 0.737			U: -0.068; p: 0.538		**U: -2.960; p: 0.003**	
Total	92.48 ± 11.78	92.16 ±	92.58 ± 11.13	92.90 ±10.95	91.93 ±	96.13 ±	91.16 ±
Mean±SD	(66-112)	10.53	(57-131)	(57-131)	10.85	11.42	10.44
(min-max)		(63-122)			(60-123)	(60-131)	(57-123)
	KW: 0.633; p: 0.954			U: 1.189; p: 0.751		**U: -5.450; p: 0.001**	

U: Mann Whitney U test., KW: Kruskall Wallis test.

Table 4. Comparison of mean scores of students from mindful eating subscales based on the products consumed (n=718)

Mindful eating questionnaire subscales	Characteristics									
	Type of bread often consumed					Frequency of fastfood consumption				
	White bread	Wheat, rye, oat bread	Whole wheat-bread	I do not eat bread	Other	I do not consume	Every-day	Once every three days	Once a week	Once a month
Disinhibition	15.41	16.18	15.79	16.28	16.70	16.36	15.91	15.30	15.21	15.94
Mean±SD	± 3.79	± 3.31	± 3.24	± 4.08	± 5.16	± 3.52	± 4.31	± 3.90	± 3.62	± 3.66
(min-max)	(5-25)	(6-24)	(9-24)	(8-24)	(9-24)	(9-24)	(6-25)	(6-25)	(5-24)	(9-25)
	KW: 1.578; p: 0.178					KW: 2.319; p: 0.056				
Emotional eating	14.94	16.03	15.91	16.08	16.40	16.42	15.46	14.96	14.87	15.37
Mean±SD	± 4.47	± 4.46	± 4.48	± 5.09	± 4.62	± 4.64	± 4.89	± 4.40	± 4.73	± 4.20
(min-max)	(5-25)	(5-25)	(5-25)	(5-25)	(9-23)	(5-25)	(5-25)	(5-25)	(5-25)	(5-25)
	KW: 2.174; p: 0.070					KW: 2.225; p: 0.065				
Eating control	13.59	13.44	13.72	14.23	13.00	13.56	14.40	13.35	13.67	13.66
Mean±SD	± 3.02	± 2.97	± 3.12	± 3.49	± 2.26	± 2.70	± 3.07	± 2.90	± 3.22	± 3.15
(min-max)	(4-20)	(7-20)	(8-20)	(4-20)	(10-17)	(7-20)	(7-20)	(4-20)	(4-20)	(7-20)
	KW: 0.887; p: 0.471					KW: 1.303; p: 0.267				
Distraction	15.23	15.21	15.20	1.45	13.90	15.01	14.67	15.07	15.20	15.73
Mean±SD	± 2.32	± 2.19	± 2.35	± 2.54	± 1.37	± 2.36	± 2.55	± 2.29	± 2.20	± 2.36
(min-max)	(8-24)	(10-19)	(10-20)	(9-21)	(11-15)	(8-20)	(9-21)	(8-21)	(9-23)	(10-24)
	KW: 0.982; p: 0.416					**KW: 3.348; p: 0.010**				
Eating discipline	11.23	12.36	12.01	11.30	9.80	11.66	9.96	11.14	11.37	12.01
Mean±SD	± 2.99	± 2.59	± 2.89	± 2.89	± 3.45	± 3.12	± 2.91	± 2.92	± 2.80	± 2.97
(min-max)	(4-20)	(6-20)	(7-18)	(6-20)	(4-16)	(5-20)	(4-17)	(4-18)	(5-19)	(4-20)
	KW: 3.641; p: 0.006					**KW: 6.132; p: 0.001**				
Awareness	14.22	14.98	14.88	15.73	15.10	15.06	14.48	14.48	14.34	14.52
Mean±SD	± 2.47	± 2.64	± 2.63	± 2.61	± 1.91	± 3.07	± 2.44	± 2.79	± 2.43	± 2.21
(min-max)	(8-22)	(10-21)	(10-22)	(11-22)	(12-18)	(9-22)	(10-19)	(8-21)	(8-21)	(8-20)
	KW: 6.996; p: 0.001					KW: 1.365; p:0.244				
Interference	6.62	6.78	6.79	7.02	6.90	7.31	6.62	6.34	6.57	6.87
Mean±SD	± 1.83	± 1.97	± 2.01	± 1.65	± 2.46	± 1.77	± 2.01	± 1.90	± 1.68	± 1.94
(min-max)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)	(2-10)
	KW: 0.861; p:0.487					**KW: 4.753; p:0.001**				
Total	91.28	95.01	94.33	96.12	91.80	95.40	91.54	90.67	91.27	94.13
Mean±SD	± 1.051	± 10.27	± 11.06	± 12.51	± 12.18	± 10.86	± 10.52	± 10.48	± 10.97	± 10.87
(min-max)	(57-122)	(73-122)	(74-129)	(70-131)	(73-108)	(63-122)	(69-116)	(57-123)	(60-122)	(66-131)
	KW: 5.019; p:0.001					**KW: 4.673; p:0.001**				

KW: Kruskall Wallis test

the number of having snacks (p<0.05). Emotional eating awareness of the students with two and three snacks was found to be high (Table 3).

Significant differences were found between their eating discipline, awareness and total scores based on the type of bread they often consumed (p<0.05). Significant differences were found between their disinhibition, emotional eating, distraction, eating discipline, interference and total scores based on eating fast food (p<0.05). Mindful eating levels were high among the students not consuming white bread and fast food (Table 4).

Discussion

Discussion of MEQ-30 scores based on descriptive characteristics

Mindful eating is a type of eating that requires focusing on the food to be consumed by noticing what, how and why the individual eats, by having a hunger-satiety awareness, by realizing his/her eating behaviors and without being affected from environmental factors.[6,7] Today, the incidence of the studies on mindful eating is increasing. Today, the studies on mindful eating has been increasing. As this issue, associated with what, how much and how is eaten, raises an awareness for food intake, the individual can enable his/her weight management. Mindful eating of the individuals can be enhanced especially at the end of interventions/trainings/therapies, and weight loss can be managed among the individuals with weight and obesity.[7,13] In this study, 15% of the students were overweight, 3.5% had a problem with obesity and their mindful eating was at a moderate level.

Gaspar et al. indicated that mindful eating was low among the adolescents, and males were less successful than the females in managing themselves about eating.[14] Females can limit themselves while eating since they

care for their physical appearance during adolescence period.[15] However, women had a high tendency for emotional eating due to the physiological and psychological needs brought by menstruation cycle.[16] In addition, control mechanisms of the women are interrupted under stress and it might become difficult for them to cope with the situation, and thus, eating control might not be managed.[17] In this study, awareness of males was found to be higher in emotional eating, eating control and awareness than the females at minimal level.

When the relationship between the age of the students and MEQ-30 was examined, a positive and significant correlation was found between disinhibition and emotional eating subscales and a negative and significant correlation between total scores from MEQ-30. The students could limit themselves in eating, and as their age increased, they were aware of the fact that seeking solution in eating was wrong under stress. However, when the other subscales were considered, their mindful eating decreased in general with increasing age due to their lack of knowledge level regarding healthy nutrition. In a study performed on university students, obesity risk was higher among the ones who had wrong dietary habits.[5] Yet, obesity risk was reported to be low among the individuals who had a high level of mindful eating due to the fewer incidences of wrong dietary habits.[18] In this study, the scores of disinhibition, emotional eating, eating control and interference subscales decreased with the increase in body mass index values of the students. In other words, students with a high body mass index could not limit themselves in eating; they could not understand the difference between hunger and satiety and exhibited engorgement behaviors. Similarly, in the study performed with 2755 university students by Moor et al., a negative correlation was reported between body mass index values of the students and their disinhibition and emotional eating scores.[19] In addition, the students with a high body mass index got a higher total and eating discipline scoresin this study. At this point, the students with a high body mass index had the knowledge of a decent diet plan and ideal amount of food for consumption in order to have a good body image, and their mindful eating level was high.

Aktaş et al. emphasized that students, who had a familial history of obesity, were under 1.27 times more risk for being overweight and for being an individual with obesity compared to the ones who did not.[20] Studies generally investigate the relationship between being an individual with obesity and familial history of obesity. However, no study was found in the literature that investigates mindful eating levels of the ones who had a person with obesity in their families. In this study, emotional eating and eating control awareness of the students who had a familial history of obesity were low. It was associated with the fact that families were role models for their children in eating as well as in all issues. Especially children who had someone in their families with obesity were considered to observe their eating behaviors and habits and learn about them, and put into practice.[21] This can be interpreted as awareness is low among the children of the parents who cannot discriminate hunger/eating desire brought by stress and who cannot control their way and routine of eating.

Discussion of MEQ-30 scores based on the students' state of physical activity

Previous studies have investigated the relationship between physical activity and eating behaviors/attitudes.[5,15,22] There is only a limited number of studies evaluating the effect of physical activity on mindful eating.[17,21] Moor et al. reported that emotional eating behaviors of the university students decreased with the increase in the amount of their physical activity; but, it did not have any effect on mindful eating.[19] Mason et al. stated that training for mindful eating was only effective when given along with physical exercise and dietary education.[23] As similar to the study by Moor et al. eating discipline, interference and mindful eating in general were found to be high among the students exercising regularly in this study.[19] They made a lot of efforts in order to manage their weight and have a good body image. At this point, mindful eating is performed with physical exercise and there is a relationship between mindful eating and physical exercise as also indicated in the study by Mason et al.[23]

Discussion of MEQ-30 scores based on eating habits of the students and the products they consumed

Risky behaviors which are hormonal and associated with psychological reasons are exhibited during adolescence period. Habits regarding eating are shown among these risky behaviors.[24] However, one of the most important criteria of mindful eating is regular diet, eating habits and eating discipline.[12] In the study by Tözün et al., it was found that 33.9% of the university students skipped a meal, ate less than three main meals per day and did not have snacks regularly.[5] Similarly, it was found in this study that the students did not have regular meals, skipped a meal and did not have snacks. When we examined the effects of the data regarding the balanced diet and their meals on the eating awareness, the students who had two and three main meals had higher subscale scores of eating discipline. In other words, the students with a regular number of main meals made their eating plans truly and had a mindful eating habit. However, whether students had regular meals or not did not have an effect on mindful eating. The reason was thought to be that students had difficulty in organizing meal hours due to the pace of school and courses and that did not have an effect on mindful eating. A significant correlation was found between the state of having snacks and emotional eat-

ing subscale of MEQ-30. Having snacks helps to struggle with insulin resistance, keeps blood glucose level at a specific level and thus, hunger is not experienced.[25] However, stress may lead to the emergence of desire for eating with the loss of hunger and satiety awareness.[26,27] Emotional eating awareness of the students who were having two and three snacks was found to be high in the study. The reason was thought to be the absence of blood glucose fluctuations.

Mindful eating level of the students with a balanced diet was found to be high in accordance with their subscale scores of disinhibition, emotional eating, eating discipline, awareness and interference and with their total score in general. Their high level of knowledge about diet, their ability to understand the difference between hunger/satiety and their awareness for focusing on the meal are the indicators of their balanced diet. Therefore, high level of mindful eating is an expected condition among the students with a balanced diet.

In the study performed with 1578 university students by Wright et al., it was reported that 40% of the students avoided to eat fatty foods such as fast food which caused obesity.[28] In the study by Altun and Kutlu, the students stated that they were consuming carbohydrates and fatty foods more although they knew protein group foods were more beneficial for health.[15] It was observed in this study that the consumption of fast food, white bread and sugary foods was also found to be high.

In the study by Tözün et al., it was found that 74.3% of the students consumed white bread and 69.8% in this current study.[5] Intensive course program and rapid pace of time were considered as the potential reasons. However, the consumption of white bread and fast food which have high glycemic index affect carbohydrate mechanism and it can be the cause and inducer of many diseases by leading to insulin resistance.[29] In this study, mindful eating levels were high among the students who were not consuming white bread and fast food; in other words, they were aware that consumption of high amount of carbohydrates was wrong.

Limitations and strengths

The only limitation of thes tudy is that the sample consists of the nursing students. The results of the study may not be open to generalization. There may be different applications in other departments such as medicine, dentist, pharmacy. The strength of this study is the sample size. The sample of the study consisted of 718 nursing students (76.0%of the universe was reached).

Conclusions

Mindful eating level increased as age increased among the nursing students in university. Sociodemographic characteristics such as sex and presence of a person with obesity in the family may affect subscales of mindful eating. Based on BMI, states of being overweight and obese were led by the lack of mindful eating and physical activity. Mindful eating level decreased as BMI increased; and besides, mindful eating level increased with physical activity. Based on these conclusions, it is suggested in this study that nutritional awareness be raised from childhood, the students with high BMI be identified, trainings be organized to make the students alter their dietary attitudes, projects be organized for healthy weight loss and for the promotion of physical activity with the partnership of Diabetes and Obesity Center and School of Physical Education in university in order to develop mindful eating.

Clinical implications

- Mindful eating level increased with the age among the nursing students in the university.
- Estimation of the relationship between mindful eating and the physical activity contributes to the decrease in obesity.
- If the nurses have the enough knowledge level about mindful eating, they could advise people about the mindful eating both in clinics and social environments.

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References

1. Basu M, Sarkar K, Shahbabu B, et al. Pattern and determinants of overweight and obesity among medical students of kolkata. Int J *Pharm Sci Res*. 2016;7(1):377-386.
2. Peltzer K, Pengpid S, Alafia Samuels T, et al. Prevalence of overweight/obesity and its associated factors among university students from 22 countries. *Int J Environ Res Publ Health*. 2014;11(7):7425-7441.
3. Webber L, Divajeva D, Marsh T, et al. The future burden of obesity-related diseases in the 53 WHO European-Region countries and the impact of effective interventions: A modelling study. *BMJ Open*. 2014;4(7):1-8.
4. Arslan SA, Daskapan A, Cakır B. Specification of nutritional and physical activity habits of university students. *TAF Prev Med Bull*. 2016;15(3):171-180.
5. Tozun M, Sozmen MK, Babaoglu AB. Association of dietary habits with obesity, physical activity and quality of life in health related faculties of a university in west Turkey. *ESTUDAM J Publ Health*. 2017;2(1):1-16.
6. Alberts HJEM, Thewissen R, Raes L. Dealing with problematic eating behaviour. The effects of a mindfulness-based intervention on eating behaviour, food cravings, dichotomous thinking and body image concern. *Appetite*. 2012;58(3):847-851.

7. Ozkan N, Bilici S. New approaches in eating behavior: Intuitive eating and mindful eating. *Gazi Univ J Health*. 2018;3(2):16-24.
8. Peitz D, Schulze J, Warschburger P. getting a deeperunderstanding of mindfulness in thecontext of eatingbehavior: Development And Validation of The Mindful Eating İnventory. *Appetite*. 2020;159:105039.
9. Tapper K, Seguias L. Theeffects of mindfuleating on foodconsumptionover a half-dayperiod. *Appetite*. 2020;145: 104495.
10. World Health Organization 2020. Orientation Program on Adolescent Health for Healthcare Providers. Availablefrom: https://www.who.int/maternal_child_adolescent/documents/pdfs/9241591269_op_handout.pdf (Date of access: 09/15/2020)
11. Adolescent Health 2020. Healthy People 2020. Available from: https://www.healthypeople.gov/2020/topics-objectives/topic/Adolescent-Health (Date of access: 09/15/2020)
12. Kose G, Tayfur M, BirinciogluI, Dönmez A. Adaptation study of the Mindful Eating Questionnaire (MEQ) into Turkish. *J Cogn Behav Psychothera Res*. 2016;3:125-134.
13. Warren JM, Smith N, Ashwell M. A structured literature review on the role of mindfulness, mindful eating and intuitive eating in changing eating behaviours: Effectiveness and associated potential mechanisms. *Nutr Res Rev*. 2017;30(2):272-283.
14. Gaspar de Matos M, Palmeira AL, Gaspar T, et al. Social support influences on eating awareness in children and adolescents: the mediating effect of self-regulatory strategies. *Glob Publ Health*. 2016;11(4):437-448.
15. Altun M, Kutlu Y. The views of adolescent about the eating behaviour: A qualitative study. *FN J Nurs*. 2015;23(3):174-184.
16. Serin Y, Sanlıer N. Emotional eating, the factors which affect food intake and basic approaches of nursing care. *J Psyc Nurs*.2018;9(2):135-146.
17. Klatzkin RR, Gaffney S, Cyrus K, et al. Stress-induced eating in women with binge-eating disorder and obesity. *Biol Psychol*. 2018;131:96-106.
18. Camilleri GM, Méjean C, Bellisle F, et al. Association between Mindfulness and weight status in a general population from the NutriNet-Santé study. *PLoS ONE*. 2015;10(6):1-14.
19. Moor KR, Scott AJ, McIntosh WD. Mindful eating and its relationship to body mass index and physical activity among university students. *Mindfulness*. 2013;4(3):269-274.
20. Aktas D, Oztürk FN, Kapan Y. Determination of the obesity prevalence and affecting risk factors, of eating habits among adolescents. *TAF Prev Med Bull*. 2015;14(5):406-412.
21. Anzman SL, Rollins BY, Birch LL. Parental influence on children's early eating environments and obesity risk: Implications for prevention. *Int J Obes*. 2010;34(7):1116-1124.
22. Choudhary K, Mathur P, Garg M, Gupta PP. Prevalence of overweight and obesity amongst adolescents and identification of risk factors. *Int J Contem Pediatr*. 2017;4(4):1153.
23. Mason AE, Epel ES, Kristeller J, et al. Effects of a mindfulness-based intervention on mindful eating, sweets consumption, and fasting glucose levels in obese adults: data from the SHINE randomized controlled trial. *J Behav Med*. 2016;39(2):201-213.
24. Kürtüncü M, Uzun M, Ayoğlu FN. The effect of adolescent training program on risky health behaviours and heatth percepiton. *J High Educ Sci*. 2015;5(2):187-195.
25. Savas HB, Gültekin F. Insulin resistance and clinical significance. *Med J Süleyman Demiral Univ*. 2017;24(3):116-125.
26. Rosenbaum DL, White KS. The relation of anxiety, depression, and stress to binge eating behavior. *J Health Psychol*. 2015;20(6):887-898.
27. Van Strien T, Cebolla A, Etchemendy E, et al. Emotional eating and food intake after sadness and joy. *Appetite*. 2013;66:20-25.
28. Wright M, Adair L, James C, et al. The association of nutrition behaviors and physical activity with general and central obesity in Caribbean undergraduate students. *Rev Panam Salud Publica*. 2015;*38*(4):278-285.
29. Dogan B, Yörük N, Oner C, et al. Evaluation of diabetes risk and eating habits of university students and personnel. *Turk J* Fam *Prac*. 2017;21(2):50-55.

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REVIEW PAPER

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Coagulation markers in diagnostic and monitoring of thromboembolic complication in COVID-19

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ABSTRACT

Introduction. Coronavirus disease 2019 (COVID-19) was first observed in China in Wuhan city, Hubei province in December, 2019, and specified as a pandemic by the World Health Organization (WHO). COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCoV2).

Aim. The aim of this article is to discuss epidemiology of thromboembolic complication in COVID-19.

Material and methods. This article is a review done in regards to discuss clinical features of the anticoagulation treatment in COVID-19.

Analysis of the literature. A review is discussed an anticoagulation treatment in 41 manuscripts.

Conclusion. Most commonly coagulation abnormalities in patient with COVID-19 is mild thrombocytopenia. Apart from their typical role in thrombosis and hemostasis, platelets mediate key aspects of immune and inflammatory.

Keywords. anticoagulation, coagulation abnormalities, COVID-19

Introduction

To date has been reported 193 097 950 cases, 4 146 985 deaths and 175 532 906 recovered. The symptomatic phase manifests generally with fever, cough and myalgia to severe respiratory failure. The diagnosis is confirmed using reverse transcriptase PCR. Laboratory abnormalities usually include: leukocytosis, leukopenia (reported in 63% of patients), neutrophilia, hypoalbuminemia, hyperglycemia and elevated liver enzymes, lactic dehydrogenase (LDH), C-reactive protein (CRP),

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ferritin, creatinine kinase, troponin and myoglobin levels. One of leading clinical features of the sever infection is a coagulopathy.[1,2] Therefore, coagulation tests may be considered useful to discriminate severe cases of COVID-19. The clinical presentation of COVID-19-associated coagulopathy is organ dysfunction primarily with high risk of venous thromboembolism., while hemorrhagic events are rarely seen.[3] Excessive inflammation, platelet activation, endothelial dysfunction, and stasis are at the root of thromboembolic complications in COVID-19.[4] COVID-19 associated alterations of hemostasis is multifactorial. Endothelial dysfunction is triggered by increased levels of von Willebrand Factor; systemic inflammation, by Toll-like receptor activation; a procoagulatory state, by tissue factor pathway activation.[5] Moreover, increased secretion of interleukins (IL-1, IL-2, IL-6, IL-7, interferon-γ inducible protein 10, MCP-1, MIP1-a), tumor necrosis factor (TNF)-alpha and, granulocyte colony stimulating factor (G-CSF) may promote lymphocyte apoptosis by cytokine storm.[6,7] Complement activation is also thought to be heavily involved in thrombosis by complex C5b-9, C3a and C5a.

Aim

We investigated the feasibility of an increase in research towards the better understanding of COVID-19.

Material and methods

All materials are based on data base such as PubMed, Science Direct and Medline.

Analysis of the literature

Epidemiology of thromboembolic complication in COVID-19

In Middeldorp et al. analysis demonstrated high risk for venous thromboembolism (VTE) in hospitalized patient with COVID-19 including intensive care units patient (ICU) on days to 2 March from 12 April 2020. Identified 199 patients because of COVID-19. One patient was excluded because he was transferred to another. Of the remaining 198 patients, 148 (75%) were hospitalized after an emergency department visit, whereas 50 (25%) were transferred from another hospital. Seventy-five patients (38%) were admitted to the ICU after being transferred from the ICU of another hospital (n=44), our general ward (n=20), or directly from the emergency department (n=11). After 7 days 39 patients (20%) were diagnosed with VTE.[8] Lodigiani et al. examined 388 patient who were admitted to a university hospital in Milan between 13.02–10.04.2020. Thromboembolic events recognized in 7.7% (28 patient) half of them were diagnosed within 24 h of hospital admission [9]. In Cui et al study 81 patients with COVID-19 in ICU. VTE proved in 20 patient 25% (25%), 8 of patients with VTE died.[10] Klok et al. confirmed VTE in 27% analyzed COVID-19 patient.[11] In a Klok' s cohort of 184 ICU patient with COVID-19 the cumulative incidence of large-vessel thrombotic events was dizzying 49%.[12] On the basis of numerous studies it has been proven that the incidence of thromboembolic complication in patients with COVID-19 may be related to poor prognosis. In this article, we present the known and used markers in the diagnosis and monitoring of thromboembolic complications in patients with COVID-19, whose knowledge of the evolution in will allow to improve diagnosis, treatment, and thus the chances of health and survival of patients with the severe form of COVID-19.

Coagulation markers in VTE in patient with COVID-19 disease

D-dimers as a the most common coagulation abnormality in COVID-19

In VTE there is an increase in the concentration of D – dimers end product of fibrin degradation by plasmin. It is not a VTE specific marker, because its high levels are found in many diseases. The cut-off point is value 500 ng/ml. Keep in mind that over 60 years of age the specificity and clinical utility of the D- dimer has increased.[13] D-dimer are sensitive (80–100%) for VTE. Therefore, normal levels rule out VTE. High level of D-dimer in COVID-19 patients associated with excess thrombin generation secondary to endothelial activation induced by the infectious, hypoxemia and microthrombosis.[14] An elevated D-dimer found in up to 45% of COVID patients, is recognized as an independent risk factor for death.[15-17] To Tang et al. research were classified 183 patients suffered from COVID-19. The changes in coagulation markers were followed from day 1 to day 14 after admission at three-day intervals. The non-survivors patients (n=21) revealed significantly higher D-dimer compared to survivors (n=162) on admission. The study has shown that existence of DIC is common in deaths. abnormal coagulation results, especially markedly elevated D-dimer and FDP, may have the potential to guide therapy and evaluate prognosis.[18] Al-Samkari based on an analysis of 400 patients stated that D-dimer >2500 ng/mL gives adjusted odds ratio for thrombosis, 6.79.[19] On the basis of tests performed in two French centers researchers concluded that at admission, D-dimer < 1.0 μg/ml has an excellent negative predictive value for VTE whereas the risk of thromboembolic events is strikingly high in patients with D-dimer level ≥ 3.0 μg/ml, which leads to the conclusion that anti-clotting prophylaxis should be dependent on the level of D-dimers.[20]

C-reactive protein (CRP)

In humans, CRP is a major acute phase protein whose concentration may increase more than 1,000-fold in severe inflammatory states. Human CRP is a pentameric protein composed of five identical non-covalently

bound subunits of 206 amino acid residues with a molecular weight of ~23 kDa.[21] The increase in inflammation markers underlying the systemic vasculitic processes and the defects in the coagulation that cause most parenchymal lesions in vital organs. CRP is an early predictor for critically COVID-19.[22] Cerebral venous thrombosis (CVT) is a unusually neurovascular emergency that has been encountered in some COVID-19 patients. Ming Tu et al. studies and 14 COVID-19 patients with CVT at the median age 43 years. The time taken from onset of COVID-19 symptoms to CVT diagnosis was a median of 7 days. A significant proportion of patients had raised D-dimer (75.0%) and CRP levels (50.0%) should be suspected in COVID-19 patients presenting with headache or seizures.[23] Wang et al. judged 19 out of the 88 COVID-19 cases who have developed deep vein thrombosis (DVT). In addition, among the 18 patients who died, 5 were diagnosed with DVT. Most of these patients had no complaint of lower limb discomfort. The blood samples for these laboratory assays were collected on the day of admission and were reviewed every 3–5 days. CRP levels on admission had positive correlations with the severity of illness and estimated 52.3-120.8mg/L.[24]

Fibrinogen

Fibrinogen is believed to play one of the key roles in the acute phase response caused by tissue damage. Following injury, exposure of activating cell surfaces or matrices activates coagulation and acute inflammation, which work together and lead to thrombin activation and conversion of fibrinogen to fibrin. Fibrinogen known as factor 1 is a glycoprotein complex made in the liver.[25] It has been proven in numerous studies on COVID-19 patients that high levels of fibrinogen positively correlate with VTE and the severe course of the disease.[26] The effect of virus has been investigated in an in vitro model panel of genes that reveal a procoagulant effect has been reported to be highly expressed in COVID-19 infected mononuclear cells, including fibrinogen (FGB, FGG).[27] As the disease recovers, fibrinogen return to normal range. Zoa et al. comparing the coagulation parameters of two groups of COVID-19 patients severe and mild. Changes in factors of coagulopathy in the severe group was higher than that in the mild group (100% vs. 66.1%). The Fibrinogen amount >7.0 g/L in 5.7% of the group of mild disease as compared to 19.1% with severe disease.[28]

Thrombocytes

Most commonly coagulation abnormalities in patient with COVID -19 is mild thrombocytopenia. Apart from their typical role in thrombosis and hemostasis, platelets mediate key aspects of immune and inflammatory.[29] Express a broad array of receptors, Toll-like receptors (TLRs), C-type lectin receptors, and nucleotide-binding and oligomerization domain–like receptors.[30] In early report from China thrombocytopenia occurred in only 12% cases.[31] Thrombocytopenia is moderate due to presence of extramedullary megakaryocytes that continuously create platelets.[32] The formation of platelets is also increased by pro-inflammatory cytokines.[33] According to the Bertolin et al. analysis there is no basis for the use of antiplatelet drugs in COVID-19 therapy. Demonstrated lower platelet reactivity (PR) was observed in patients with COVID-19 in comparison with healthy individuals. The rates of low PR were 27.5% in the COVID-19 group and 21.7% in the control group.[34]

IL-6

As already mentioned, one of the VTE factors is a cytokine storm accompanied by, among others, an increase in IL-6 or IL-1 values which cause thrombosis by activating platelets, endothelium, monocytes, and the factor VIIa pathway.[35] In Ranucci et al. study IL-6 levels were measured at the admission in the ICU in a 16 patient with COVID-19. All the patients had elevated level of IL-6, and a clear association between IL-6 and fibrinogen was demonstrated.[36]

Homocysteine

A high level of homocysteine in the makes a person more prone to endothelial cell injury, which leads to inflammation in the blood vessels, which in turn may lead to atherogenesis, which can result in ischemic injury In the last decade, epidemiological observations have pointed towards a plausible association between hyperhomocysteinemia and nervous system neurodegenerative disorders.[37] Yang et al. and Ponti et al. observed positive correlation between its level and pulmonary embolism and VTE.[38,39] Moreover, in COVID-19 with VTE patients it positively correlates with D-dimers.[40]

Other coagulation factors

Helms et al. have made multicenter prospective cohort study in ICU COVID-19 patient. 150 COVID-19 patients were analyzed. Sixty-four clinically relevant thrombotic complications were diagnosed in 150 patients, mainly pulmonary embolisms (16.7%). 28/29 patients (96.6%) receiving continuous renal replacement therapy experienced circuit clotting. Three thrombotic occlusions (in 2 patients) of centrifugal pump occurred in 12 patients (8%) supported by ECMO. Apart from the indicators mentioned in the article, they also dealt with von Willebrand factor (vWF) activity von Willebrand factor antigen (vWF:Ag) and lupus anticoagulant. vWF activity and vWF: Ag were considerably increased, as well as factor VIII. Also 50 patients out of the 57 tested (87.7%) had positive lupus circulating anticoagulant during their hospitalization.[41]

Conclusion

Anticoagulation treatment in COVID-19 patients presents a major challenge due to the various mechanisms that must be overcome, because of Considering the multi-pathogenesis of thromboembolic complication in COVID-19 patients. Although we currently know of many anticoagulant treatment regimens in COVID-19, there are still no uniform guidelines for the treatment of patients with thromboembolic complications.

References

1. Hadid T, Kafri Z, Al-Katib A. Coagulation and anticoagulation in COVID-19. *Blood Rev.* 2021;47:100761.
2. Beun R, Kusadasi N, Sikma M, Westerink J, Huisman A. Thromboembolic events and apparent heparin resistance in patients infected with SARS-CoV-2. *Int J Lab Hematol*. 2020;42(1):19-20.
3. Alsharif W, Qurashi A. Effectiveness of COVID-19 diagnosis and management tools: A review. *Radiography (Lond)*. 2021;27(2):682-687.
4. Bikdeli B, Madhavan MV, Jimenez D, et al. Global COVID-19 Thrombosis Collaborative Group, Endorsed by the ISTH, NATF, ESVM, and the IUA, Supported by the ESC Working Group on Pulmonary Circulation and Right Ventricular Function. COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. *J Am Coll Cardiol*. 2020;75(23):2950-2973.
5. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. *J Clin Virol*. 2020;127:104362.
6. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, et al. Hematological findings and complications of COVID-19. *Am J Hematol*. 2020;95(7):834-847.
7. Liao YC, Liang WG, Chen FW, Hsu JH, Yang JJ, Chang MS. IL-19 induces production of IL-6 and TNF-alpha and results in cell apoptosis through TNF-alpha. *J Immunol*. 2002;169(8):4288-4297.
8. Middeldorp S, Coppens M, van Haaps TF, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. *J Thromb Haemost*. 2020;18(8):1995-2002.
9. Lodigiani C, Iapichino G, Carenzo L. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Humanitas COVID-19 Task Force *Thromb Res.* 2020;191:9-14.
10. Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. *J Thromb Haemost* 2020;18:1421-1424.
11. Klok FA, Kruip MJHA, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res.* 2020;191:145-147.
12. Klok FA, Kruip MJHA, Van Der Meer NJM, et al. Confirmation of the high cumulative incidence of thrombotic complications in critically ill ICU patients with COVID-19: an updated analysis. *Thromb Res.* 2020;191:148-150.
13. Chojnowski K. Dimer D – diagnostyczny i rokowniczy marker żylnej choroby zakrzepowo-zatorowej. *Hematologia*. 2010;1,2:102-108.
14. Marchandot B, Sattler L, Jesel L, et al. COVID-19 Related Coagulopathy: A Distinct Entity? *J. Clin. Med.* 2020;9:1651.
15. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395:1054-1062.
16. Li Y, Zhao K, Wei H, et al. Dynamic relationship between D-dimer and COVID-19 severity. *Br J Haematol*. 2020;190(1):e24-e27.
17. Li C, Hu B, Zhang Z, et al. D-dimer triage for COVID-19. *Acad Emerg Med*. 2020;27(7):612-613.
18. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost.* 2020;18(4):844-847.
19. Al-Samkari H, Karp Leaf RS, Dzik WH, et al. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood.* 2020;136(4):489-500.
20. Artifoni M, Danic G, Gautier G, et al. Systematic assessment of venous thromboembolism in COVID-19 patients receiving thromboprophylaxis: incidence and role of D-dimer as predictive factors. *J Thromb Thrombolysis* 2020;50(1):211-216.
21. Pathak A, Agrawal A. Evolution of C-Reactive Protein. *Front Immunol*. 2019;10:943.
22. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. *Crit Rev Clin Lab Sci*. 2020;57(6):389-399.
23. Tu TM, Goh C, Tan YK, et al. Cerebral Venous Thrombosis in Patients with COVID-19 Infection: a Case Series and Systematic Review. *J Stroke Cerebrovasc Dis*. 2020;29(12):105379.
24. Wang W, Sun Q, Bao Y, et al. Analysis of Risk Factors for Thromboembolic Events in 88 Patients with COVID-19 Pneumonia in Wuhan, China: A Retrospective Descriptive Report. *Med Sci Monit.* 2021;27:e929708.
25. Erdoes G, Koster A, Meesters MI, et al. The role of fibrinogen and fibrinogen concentrate in cardiac surgery: an international consensus statement from the Haemostasis and Transfusion Scientific Subcommittee of the European Association of Cardiothoracic Anaesthesiology. *Anaesthesia.* 2019;74(12):1589-1600.
26. Thachil J, Agarwal S. Understanding the COVID-19 coagulopathy spectrum. *Anaesthesia.* 2020;75(11):1432-1436.
27. Ng LF, Hibberd ML, Ooi EE, et al. A human in vitro model system for investigating genome-wide host responses to SARS coronavirus infection. *BMC Infect Dis.* 2004;4:34.
28. Zou Y, Guo H, Zhang Y. Analysis of coagulation parameters in patients with COVID-19 in Shanghai, China. *Biosci Trends*. 2020;14(4):285-289.

29. Manne BK, Denorme F, Middleton EA, et al. Platelet gene expression and function in patients with COVID-19. *Blood.* 2020;136(11):1317-1329.
30. Semple JW, Italiano JE Jr., Freedman J. Platelets and the immune continuum. *Nat Rev Immunol.* 2011;11(4):264-274.
31. Connors JM, Levy JH. COVID-19 an its implications for thrombosis and anticoagulation. *Blood.* 2020;135(23): 2033-2040.
32. Becker RC. COVID-19 update: Covid-19-associated coagulopathy. *J Thromb Thrombolysis*. 2020;50(1):54-67.
33. Iba T, Levy JH, Levi M, Thachil J. Koagulopatia w COVID-19. *J Thromb Haemost*. 2020;18:2103-2109.
34. Bertolin AJ, Dalçóquio TF, Salsoso R, et al. Platelet Reactivity and Coagulation Markers in Patients with COVID-19. *Adv Ther*. 2021;38(7):3911-3923.
35. Mucha SR, Dugar S, McCrae K, et al. Coagulopathy in COVID-19: manifestations and management. *Cleve Clin J Med*. 2020;87(8):461-468.
36. Ranucci M, Ballotta A, Di Dedda U, et al. The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. *J. Thromb. Haemost*. 2020;18(7):1747-1751.
37. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. *Nutr J.* 2015;14:6.
38. Ponti G, Ruini C, Tomasi A. Homocysteine as a potential predictor of cardiovascular risk in patients with COVID-19. *Med Hypotheses.* 2020;143:109859.
39. Yang X, Yang Q, Wang Y, et al. Thrombocytopenia and its association with mortality in patients with COVID-19. *J Thromb Haemost.* 2020;18(6):1469-1472.
40. Todua F, Akhvlediani M, Vorobiova E, Tsivtsivadze G, Baramidze A. Homocysteine and D-dimer levels and multilayer computed tomography for diagnosing pulmonary artery thromboembolism. *Vessel Plus.* 2017;1:38-42.
41. Helms J, Tacquard C, Severac F, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med.* 2020;46(6):1089-1098.

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REVIEW PAPER

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The significance of glycocalyx in medicine

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ABSTRACT

Introduction. The glycocalyx is a gel-like layer covering the membrane of many cells, especially cells of epithelial tissue. It consists of membrane-bound proteoglycans, glycosaminoglycan chains, glycoproteins, and adjacent proteins. Glycocalyx is necessary in maintaining the permeability of vessels, modulation of inflammatory responses and interactions between cells. It is also involved in cell adherence, mobility, mechanotransduction, regulation of the cell cycle and cell. Abnormalities in the structure and function of the glycocalyx underlie many diseases and disorders such as dry eyes disease, diabetes and its complications as well as sepsis.

Aim. In this review, we present the current view on the role of glycocalyx in human diseases.

Material and methods. This review was performed according to latest literature from the following databases: EBSCO, PubMed, Science Direct, and Springer Link.

Analysis of the literature. Pathological mechanisms such as disruption of the glycocalyx barrier and decreased hydration of the ocular epithelial surface cause dry eye disease. During hyperglycaemia, glycocalyx dysfunction occurs, which leads to its dysfunction and activation of the prothrombotic system. Moreover, the increase in the concentration of hyaluronidase leads to increase in the plasma hyaluronan levels and promotion of endothelial dysfunction. Additionally, degradation of glycocalyx in sepsis prevails over increased synthesis of its components strongly favors its enhanced enzymatic degradation.

Conclusion. A better understanding of glycocalyx impairment in disease could alter therapeutic strategies to improve patient outcomes.

Keywords. disease, glycocalyx degradation, glycosaminoglycans, hyperglycemia, medicine

Introduction

The glycocalyx is a carbohydrate-rich layer that covers the membrane of many cells, especially cells of epithelial tissue. It was first described on the surface of endothelial cells by Danielli in 1940.[1] Glycocalyx is necessary in maintaining the proper permeability of blood vessels and modulates interactions between blood cells and endothelium, and is also involved in cell adherence, mobility, mechanotransduction, regulation of the cell cycle and proliferation of normal cells.[2] Moreover, glycocalyx

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participates in immunological processes by modulating inflammatory responses by binding cytokines and weakening their binding to cell membrane receptors. In addition, it is known to be involved in the process of angiogenesis and metastasis.[3]

The biochemical composition of glycocalyx depends on the type of cell, type of tissue and the prevailing mechanical and physicochemical conditions, but its main building blocks are proteoglycans and glycoproteins. Proteoglycans have long and unbranched carbohydrate chains consisting of core proteins that covalently link to at least one molecule of glycosaminoglycans (GAG). GAGs are linear polysaccharides made up of repeating disaccharide units with N-acetylated or N-sulfated hexosamine or uronic acid (glucuronic acid or iduronic acid) or galactose. The dominant GAG group is heparan sulfate, which has numerous sulfate groups, which accounts for 50–90% of GAG. The second most common GAG in the glycocalyx of endothelial cell is chondroitin sulfate/dermatan sulfate.[4] The presence of heparan sulfate and chondroitin sulfate is estimated to have a typical ratio of 4:1 for the endothelium.[5] Proteoglycans show great structural diversity as they can contain GAGs of various numbers and chain lengths, which are further modified by repeating disaccharides forming a complex pattern of sulfate groups and a different core protein structure. The core proteins of proteoglycans, mainly syndecan and glypican, are linked to the cell membrane by a glycosylphosphatidylinositol anchor or a transmembrane protein domain.[6] On the other hand, glycoproteins are composed of a protein to which usually straight-chain oligosaccharides are covalently attached, usually consisting of several monosaccharide residues (N-acetylhexosamine, galactose or mannose). The major glycoproteins of the glycocalyx include selectins, integrins, and other adhesion molecules such as intercellular adhesion molecules 1 and 2, and thrombocyte/endothelial adhesives.[7,8] Additionally, other molecules are attached to the glycocalyx, including growth factors and their receptors, coagulation inhibitors, lipoprotein lipase and low-density lipoproteins, and plasma and extracellular matrix proteins.[6] Research by Kabedev and Lobaskin shows that changes in glycocalyx glycan density determine its resistance to slight deformation, which also prevents stress transfer to the cell membrane.[9]

The thickness of the glycocalyx layer also depends on the type of cell and the diameter of the vessels, but measurements with an atomic force microscope revealed that on the surface of the endothelium the structure is about 0.3–1 μm.[10] Nevertheless, Ebong et al. using transmission electron microscopy observed that the glycocalyx on the surface of the endothelium lining the bovine aorta can be as much as 11 μm thick.[11]

Pathological breakdown of glycocalyx occurs in response to many factors including mechanical cell stress, endotoxins, pro-inflammatory cytokines such as tumor necrosis factor alpha, reactive oxygen species, and hyperglycemia.[12,13] Abnormalities in the structure and functioning of the glycocalyx underlie many diseases and disorders such as dry eyes disease, diabetes and its complications as well as sepsis.

Aim

In this review, we present the current view on the role of glycocalyx in human diseases

Material and methods

This review was performed according to latest literature from the following databases: EBSCO, PubMed, Science Direct, and Springer Link.

Analysis of the literature

Dry eyes disease

Dry eye disease is a common and multifactorial disease with a high prevalence worldwide. In the apical part of the eye epithelium there are microfolds covered with glycocalyx, whose main function is to increase the surface area. Glycocalyx protects the surface of epithelial cells against chemical and mechanical damage, and also prevents the penetration of pathogens into the eye, reduces friction during blinking and maintains the hydrophilic surface of the eye. Pathological mechanisms such as disruption of the glycocalyx barrier and decreased hydration of the ocular epithelial surface cause dry eye disease.[14]

Research indicates that transmembrane mucins and galectin-3 play a key role in the glycocalyx epithelial barrier and are essential for maintaining proper hydration of the eye epithelium. The very large glycans in transmembrane mucus are necessary to keep the eye surface wettable due to their very high water-holding capacity. Among them, three main mucins can be distinguished – MUC1, MUC4 and MUC16. *In vitro* studies show that MUC1 serves as an anti-adhesion and signaling molecule. In turn, MUC4 is involved in proliferation signaling by activating the ErbB2 tyrosine kinase receptor. MUC16, which is the largest membrane-bound mucin, plays a key role in the formation of the protective shell.[15,16] It has also been shown that the reduction or alteration of the chemical composition of the glycocalyx on the surface of the eye may be one of the factors contributing to the dryness of the eyes associated with the use of soft contact lenses.[17]

Diabetes and its complications

One of the diseases in which the glycocalyx plays an important and confirmed role is diabetes. With this disease, there is a marked increase in the risk of developing vascular complications. Vascular complications developing on a micro and macro scale lead to a deterioration of the comfort and quality of life as well as a shortened life expectancy of patients.[18] An early symptom of dam-

age to blood vessels is an increase in their permeability, which results in the disruption of their physiological functions.[19,20] This leads to the onset of albuminuria and the development of cardiac complications.[12] Under physiological conditions, the glycocalyx protects cells from direct contact with blood. During hyperglycaemia, glycocalyx dysfunction occurs, which leads to its dysfunction and activation of the prothrombotic system.[21] In patients with type 1 diabetes, the volume of glycocalyx is reduced by 50% within 6 h after induction of acute hyperglycaemia.[22] The loss of glycocalyx is directly related to the increase in the concentration of hyaluronidase what leads to increase in the plasma hyaluronan levels and promotion of endothelial dysfunction.[23] Moreover, hyperglycemia changes the phenotype of fibroblasts which are more elongated but less motile and less contractile than healthy dermal fibroblasts. It is caused by formation of larger focal adhesions stabilized by a glycocalyx as well as associated with increased expression of the cell surface glycoprotein MUC16.[24] Additionally, recent study by Wadowski et al. indicated that the loss of glycocalyx dimensions is correlated with increasing level of glycated hemoglobin.[25]

Research was conducted to confirm the hypothesis of possible beneficial effects that restoration of glycocalyx volume may have in the context of preventing the development of diabetes complications. It was examined whether there was a reduction in the amount of glycocalyx in patients with type 2 diabetes and whether oral administration of glycosaminoglycan precursors improved the activity of glycocalyx. Ten men with type 2 diabetes and ten control group were included in the study, in whom the level of glycocalyx was determined before and 2 months after the administration of one of GAGs – sulodexide at a dose of 200 mg/day. The conducted research has shown that in the case of patients with type 2 diabetes, the activity of glycocalyx in relation to healthy people was impaired. This group of patients also experienced an increase in vascular permeability (increased albuminuria).[26] GAGs such as sulodexide act at multiple levels: they promote glycocalyx reconstitution, control glycocalyx degrading enzymes, exert anti-inflammatory effects and have anti-apoptotic and anti-senescence effects on endothelial cells.[27]

The kidneys of people with diabetes are characterized by glomerular hypertrophy, thickening of the basement membrane, and damage to endothelial cells and glycocalyx. Even in the case of microalbuminuria, a reduced amount of glycocalyx and the enzyme heparinidase, responsible for the breakdown of GAGs, were engraved.[28,29]

The hyperglycemia present in diabetes causes the production of reactive oxygen species as well as vascular endothelial growth factor and angiotensin II.[30,31] These substances reduce the selectivity of the barrier as a result of the weakening of the action of superoxide dismutase, the function of which is the decomposition of the superoxide anion. Such oxidative stress damages the glycocalyx and impairs filtration.[32] As the disease progresses, the amount of protein in the urine increases – podocytes are damaged, and angiotensin II, along with increased glucose levels, weakens the production of proteoglycans by podocytes.[33-35] More and more active nephrons are lost, causing the remaining nephrons to become overloaded and hypertrophied, followed by interstitial tissue fibrosis and renal dysfunction.[33] As a result, anemia, secondary hyperparathyroidism, bone mineralization disorders, and soft tissue calcification appear.[36]

In improving the health of people with cardiovascular diseases, related to type II diabetes, exercise and a low-salt diet can help. This helps to maintain a "thick layer" of the endothelial glycocalyx which is important in preventing clotting.[37] With the increase of physical activity, increases heart rate, and the rate of blood flow in blood vessels. This favors the transport of Na^+ ions from the endothelial glycocalyx. Due to the action of electrostatic forces, the space between red blood cells, the glycocalyx of the endothelium, increases.[38,39] The charge of erythrocytes is negative, and along with the removal of sodium cations (supplied, among others, with NaCl salt) from the glycocalyx, the total charge is more negative and repulsion increases.[40]

Sepsis

Sepsis is an ominous clinical manifestation of a severe generalized infection leading to systemic multiorgan sequelae such as hypotension, acute respiratory distress syndrome, renal failure, and lactic acidosis, among others. Glycocalyx is also used effectively as a biomarker for sepsis. During the initial development of sepsis, it is damaged.[41,42] The fact that the degradation of glycocalyx in sepsis prevails over increased synthesis of at least one of its components strongly favors its enhanced enzymatic degradation. It is carried out by sheddases, a disintegrin and metalloproteinases, matrix metalloproteinase, heparanase-1, and hyaluronidases.[43-45] As a result, the level of circulating glycocalyx components such as syndecans, heparan sulfates and hyaluronic acid increases. Their level is measured, on the basis of which the developing pathogenic process can be determined. Despite the accumulated knowledge regarding the important roles of the glycocalyx, the relationship between derangement of the endothelial glycocalyx and severity of sepsis has not been adequately elucidated.[46]

Conclusion

Over the past two decades, glycocalyx has attracted more and more attention from scientists, and its role in health and disease has been intensively studied. However, despite the well-described pathological sequelae that follow glycocalyx damage, there are few reports of therapeutic strategies targeting impairment of the glycocalyx structure and function.

References

1. Danielli JF. Capillary permeability and oedema in the perfused frog. *J Physiol.* 1940;98:109-129.
2. Kapellos GE, Alexiou TS. Chapter 1 - Modeling Momentum and Mass Transport in Cellular Biological Media: From the Molecular to the Tissue Scale. *Transport in Biological Media* 2013:1-40.
3. Kang H, Wu Q, Sun A, Liu X, Fan Y, Deng X. Cancer Cell Glycocalyx and Its Significance in Cancer Progression. *Int J Mol Sci.* 2018;19(9):2484.
4. Bar P, Galiniak S, Bartusik-Aebisher D, et al. Glycosaminoglycan concentration in cancer tissue. *Eur J Clin Exp Med.* 2019;17(2):175-178.
5. Mulivor AW, Lipowsky HH. Inflammation- and ischemia--induced shedding of venular glycocalyx. *Am J Physiol Heart Circ Physiol.* 2004;286(5):H1672-H1680.
6. Kolářová H, Ambrůzová B, Svihálková Šindlerová L, Klinke A, Kubala L. Modulation of endothelial glycocalyx structure under inflammatory conditions. *Mediators Inflamm.* 2014;2014:694312.
7. Yilmaz O, Afsar B, Ortiz A, Kanbay M. The role of endothelial glycocalyx in health and disease. *Clin Kidney J.* 2019;12(5):611-619.
8. Tarbell JM, Cancel LM. The glycocalyx and its significance in human medicine. *J Intern Med.* 2016;280(1):97-113.
9. Kabedev A, Lobaskin V. Structure and elasticity of bush and brush-like models of the endothelial glycocalyx. *Sci Rep.* 2018;8(1):240.
10. Bai K, Wang W. Spatio-temporal development of the endothelial glycocalyx layer and its mechanical property in vitro. *J R Soc Interface.* 2012;9(74):2290-2298.
11. Ebong EE, Macaluso FP, Spray DC, Tarbell JM. Imaging the endothelial glycocalyx in vitro by rapid freezing/freeze substitution transmission electron microscopy. *Arterioscler Thromb Vasc Biol.* 2011;31(8):1908-1915.
12. Butler MJ, Down CJ, Foster RR, Satchell SC. The Pathological Relevance of Increased Endothelial Glycocalyx Permeability. *Am J Pathol.* 2020;190(4):742-751.
13. Hahn RG, Patel V, Dull RO. Human glycocalyx shedding: Systematic review and critical appraisal. *Acta Anaesthesiol Scand.* 2021;65(5):590-606.
14. Uchino Y. The Ocular Surface Glycocalyx and its Alteration in Dry Eye Disease: A Review. *Invest Ophthalmol Vis Sci.* 2018;59(14):DES157-DES162.
15. Ablamowicz AF, Nichols JJ. Ocular Surface Membrane--Associated Mucins. *Ocul Surf.* 2016;14(3):331-341.
16. Portal C, Gouyer V, Gottrand F, Desseyn JL. Ocular mucins in dry eye disease. *Exp Eye Res.* 2019;186:107724.
17. Fukui M, Yamada M, Akune Y, Shigeyasu C, Tsubota K. Fluorophotometric Analysis of the Ocular Surface Glycocalyx in Soft Contact Lens Wearers. *Curr Eye Res.* 2016;41(1):9-14.
18. Dogné S, Flamion B, Caron N. Endothelial Glycocalyx as a Shield Against Diabetic Vascular Complications: Involvement of Hyaluronan and Hyaluronidases. *Arterioscler Thromb Vasc Biol.* 2018;38(7):1427-1439.
19. Parsons JT, Horwitz AR, Schwartz MA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat Rev Mol Cell Biol.* 2010;11(9):633-643.
20. Weinbaum S, Cancel LM, Fu BM, Tarbell JM. The Glycocalyx and Its Role in Vascular Physiology and Vascular Related Diseases. *Cardiovasc Eng Technol.* 2021;12(1):37-71.
21. Cao RN, Tang L, Xia ZY, Xia R. Endothelial glycocalyx as a potential theriapeutic target in organ injuries. *Chin Med J (Engl).* 2019;132(8):963-975.
22. Nieuwdorp M, van Haeften TW, Gouverneur MCLG, et al. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. *Diabetes.* 2006;55:480-486.
23. Nieuwdorp M, Mooij HL, Kroon J, et al. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. *Diabetes.* 2006;55(4):1127-1132.
24. Jahan I, Pandya J, Munshi R, Sen S. Glycocalyx disruption enhances motility, proliferation and collagen synthesis in diabetic fibroblasts. *Biochim Biophys Acta Mol Cell Res.* 2021;1868(4):118955.
25. Wadowski PP, Kautzky-Willer A, Gremmel T, et al. Sublingual microvasculature in diabetic patients. *Microvasc Res.* 2020;129:103971.
26. Broekhuizen LN, Lemkes BA, Mooij HL, et al. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. *Diabetologia.* 2010;53(12):2646-2655.
27. Masola V, Zaza G, Onisto M, Lupo A, Gambaro G. Glycosaminoglycans, proteoglycans and sulodexide and the endothelium: biological roles and pharmacological effects. *Int Angiol.* 2014;33(3):243-254.
28. Mahtal N, Lenoir O, Tharaux PL. Glomerular Endothelial Cell Crosstalk With Podocytes in Diabetic Kidney Disease. *Front Med (Lausanne).* 2021;8:659013.
29. van der Vlag J, Buijsers B. Heparanase in Kidney Disease. *Adv Exp Med Biol.* 2020;1221:647-667.
30. Meza CA, La Favor JD, Kim DH, Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? *Int J Mol Sci.* 2019;20(15):3775.
31. Fakhruddin S, Alanazi W, Jackson KE. Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury. *J Diabetes Res.* 2017;2017:8379327.
32. Jourde-Chiche N, Fakhouri F, Dou L, et al. Endothelium structure and function in kidney health and disease. *Nat Rev Nephrol.* 2019;15(2):87-108.
33. Singh A, Ramnath RD, Foster RR, et al. Reactive oxygen species modulate the barrier function of the human glomerular endothelial glycocalyx. *PLoS One.* 2013;8(2):e55852.
34. Fu J, Lee K, Chuang PY, Liu Z, He JC. Glomerular endothelial cell injury and cross talk in diabetic kidney disease. *Am J Physiol Renal Physiol.* 2015;308(4):F287-297.
35. Ebefors K, Lassén E, Anandakrishnan N, Azeloglu EU, Daehn IS. Modeling the Glomerular Filtration Barrier and Intercellular Crosstalk. *Front Physiol.* 2021;12:689083.

36. Shroff R. Reducing the burden of cardiovascular disease in children with chronic kidney disease: prevention vs. damage limitation. *Pediatr Nephrol.* 2021. doi: 10.1007/s00467-021-05102-y.
37. Kröpfl JM, Beltrami FG, Rehm M, Gruber HJ, Stelzer I, Spengler CM. Acute exercise-induced glycocalyx shedding does not differ between exercise modalities, but is associated with total antioxidative capacity. *J Sci Med Sport.* 2021;24(7):689-695.
38. Majerczak J, Grandys M, Duda K, et al. Moderate-intensity endurance training improves endothelial glycocalyx layer integrity in healthy young men. Exp Physiol. 2017;102(1):70-85.
39. Schmitz B, Niehues H, Lenders M, et al. Effects of high-intensity interval training on microvascular glycocalyx and associated microRNAs. *Am J Physiol Heart Circ Physiol.* 2019;316(6):H1538-H1551.
40. Oberleithner H, Peters W, Kusche-Vihrog K, et al. Salt overload damages the glycocalyx sodium barrier of vascular endothelium. *Pflugers Arch.* 2011;462(4):519-528.
41. Wiesinger A, Peters W, Chappell D, et al. Nanomechanics of the endothelial glycocalyx in experimental sepsis. *PLoS One.* 2013;8(11):e80905.
42. Nieuwdorp M, Meuwese MC, Mooij HL, et al. Tumor necrosis factor-alpha inhibition protects against endotoxin-induced endothelial glycocalyx perturbation. *Atherosclerosis.* 2009;202(1):296-303.
43. Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. *Crit Care*. 2019;23(1):16.
44. Ince C, Mayeux PR, Nguyen T, et al. The endothelium in sepsis. *Shock.* 2016;45(3):259-270.
45. Goligorsky MS, Sun D. Glycocalyx in Endotoxemia and Sepsis. *Am J Pathol.* 2020;190(4):791-798.
46. Iba T, Levy JH. Derangement of the endothelial glycocalyx in sepsis. *J Thromb Haemost.* 2019;17(2):283-294.

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REVIEW PAPER

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New fluorescent imaging technics in gastrology

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ABSTRACT

Introduction. There is a need to develop a new imaging technique in medicine. Gastroenterology is the branch of medicine focused on the digestive system and its disorders therefore for this branch is needed to detect all problems affecting the gastrointestinal tract.

Aim. The aim of this article is to complete discuss the possibility of the new fluorescent imaging technics in gastrology to use innovative screening to identify individuals at an early stage.

Material and methods. We discuss here imaging techniques such as include x-rays, computed tomography, scans, and magnetic resonance imaging in gastrology. Spectroscopy is the study of the formation and interpretation of spectra resulting from the interaction of all types of radiation on matter understood as a community of atoms and molecules. Various spectroscopic techniques are obtained by combining different types of radiation with different ways of its interaction with the test sample. They provide the opportunity to obtain detailed information about the tested substance – from its atomic composition, through its chemical structure, to its surface structure.

Analysis of the literatue. The tissue fluorescence spectrum can be obtained by: (1) autofluorescence, or natural or primary fluorescence, i.e. by direct irradiation of the tissue with laser radiation (laser-induced fluorescence – LIF) and (2) photodynamic diagnosis (PDD), where spectrum analysis is preceded by systemic or local administration of the photosensitizer.

Conclusion. The use of fluorescence imaging in colon cancer patient has potential to improve quality of treatment and diagnosis.

Keywords. diagnostics, fluorescent imaging, gastrology

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Introduction

It is estimated that, in Europe in 2020, colorectal cancer accounted for 12.7% of all new cancer diagnoses and 12.4% of all deaths due to cancer. That made it the second most frequently occurring cancer (after breast cancer) and the second cause of cancer death (after lung cancer).[1] Screening, used to identify individuals at an early stage, has improved outcome therefore, is increasingly used treatment of minimally invasive surgery, where preoperative endoscopic marking is necessary.[2] Most colorectal cancer develop from colorectal adenomas, and colonoscopy is regarded as the gold standard method for both detection and resection of such lesions[3] Tumours in colonoscopy are described in relation to major anatomic landmarks such as the ileocecal valve, hepatic or splenic flexure, but these make anatomical measurements challenging and inaccurate. If the surgeon anticipates intraoperative localization may be difficult, lesions other than rectal or cercal ones should probably be marked by preoperative tattooing.[4] In the context of laparoscopic colorectal resection, preoperative endoscopic tattooing is a safe and reliable method of tumor localization in most cases. Localizing colon and proximal rectal lesions with tattoos may be preferable to other localization techniques including intraoperative endoscopy.[5]

Aim

We investigated the feasibility of a fluorescence imaging technique using light-emitting diode (LED)-activated indocyanine green (ICG) fluorescence in gastroentrology.

Material and methods

According to the recommendations, gastrointestinal diseases, neoplasms and neuroendocrine tumours have been investigated in data base such as PubMed, Science Direct and Medline. This paper cover only newest technological solution for fluorescent imaging.

Analysis of the literature

As early as 1958, Sauntry and Knudtson first introduced the idea of tattooing a colonic polyp using methylene blue.[6] But in 1975, when Ponsky and King suggested that endoscopic tattooing could be useful for intraoperative localisation of colonic lesions, that the technique became more widely used. [7] From initial studies, it was demonstrated that India ink is the most effective agent based on permanence and limited biologic reaction the tattoo dye.[8,9]

Since the colonoscopist cannot reliably predict which portion of the bowel wall will be best visualized at the time of laparotomy, Hymana i Waye'a have proposed the use of a "four quadrant" circumferential tattooing in the colon, which is now used.[10] Fu et al. introduced conventional technique, where saline where injected into the submucosal layer and next the syringe of saline was replaced by another syringe containing India ink and injected, next India ink syringe was replaced by the first syringe of saline and added saline to push out India Ink.[11] Unfortunately, this dye produces a permanent and easily visible staining, complications such as abscess formation, focal peritonitis, inflammatory tumours, idiopathic inflammatory bowel disease. Current practices are variable and are operator-dependent. There are no evidence-based guidelines to aid endoscopists in clinical practice. Despite the National Bowel Cancer Screening Programme guidelines, a significant proportion of colorectal lesions are still not tattooed during their first endoscopy or they are tattooed in wrong place.[12] Researchers are looking for new ways of tattooing. An alternative to tattooing is the endoscopic placement of endoclips adjacent to the lesion, which can then be localized using intraoperative fluoroscopy or used preoperative endoscopic tattooing using indocyanine green (ICG), an exogenous fluorophore visible in real time using near-infrared (NIR) cameras.

Indocyanine green is an amphiphilic, tricarbocyanine iodide dye that is reconstituted in aqueous solution of pH 6.5 for intravenous injection in patients. Indocyanine green fluorescence has high contrast and sensitivity because the near-infrared light used to measure fluorescence makes tissues appear more translucent, probing several millimetres into the tissue, than at other wavelengths. The molecule is generally excited between 750 and 800 nm, and fluorescence is viewed around the maximum peak of 832 nm.[13] Ushimaru et al retrospectively analysed preoperative and perioperative data from consecutive gastric cancer patients undergoing elective laparoscopic distal gastrectomy and concluded ICG fluorescence imaging are feasible and safe and could potentially serve as a tumour marker to determine the surgical resection line.[14] For example Tanaka et al was injected Indocyanine green into the submucosa layer of the stomach approximately 1 cm away from the tumour edge by using an endoscopic puncture needle 1 or 3 days before surgery and next used laparoscopic system for ICG detection. The imaging was generated by a high-end full high-definition camera system connected to a laparoscope with a 30 0 field of direction and a powerful xenon light source and then detecting indocyanine green identified the tumour location.[15] Nagata et al injected India ink near the lesion and then ICG was injected on the other side of the lesion. During subsequent laparoscopic colorectal resection, the colon and rectum were first observed with white light. Then, ICG was activated with a near-infrared LED at 760nm as the light source with a laparoscopic imaging system [16]. Ozawa et al made local injection of 0.5-mL 0.25% ICG solution in the submucosal layer around the tumour in the large intestine concomitantly with normal India ink injec-

tion (0.2 mL). Identification of the tumour site during surgery was achieved with the PINPOINT® endoscopic fluorescence imaging system, which was developed using a SPYTM small-vessel image data-processing system. During laparoscopic surgery, tumour sites were observed with the help of visible and fluorescent light.[17] Wanatanabe made indocyanine green injections undergoing preoperative colonoscopy for early colon cancer or colon adenoma. During subsequent laparotomy, the colon was first observed with the naked eye, and then using a prototype machine with a charge-coupled device (CCD) video camera equipped with a cut-off filter and a LED at a wavelength of 760 nm as the light source.[18] Different method was presented by Barberio et al. They used the fluorescent coating material (near-infrared coating of equipment: NICE) which was synthesized by combining a biocompatible polymer poly(methyl methacrylate) (PMMA) with a specifically engineered fluorescent dye. This fluorescent paint can coat medical instruments using direct immersion or a paintbrush. In six pigs, they made tattoos using 0.05 mg/mL of Indocyanine Green and marked regions using the novel fluorescently coated clips. Simultaneously, NIR laparoscopy was executed. During laparoscopy, fluorescence intensity was assessed. In one human cadaver, FOSC was used to mark a site on the stomach and on the sigmoid colon, respectively. Intraoperative detection during NIR laparoscopy was assessed.[19] Choi et al used the fluorescent clips which were placed on the mucosal surface of a porcine colon and stomach, and the operator then attempted to identify the fluorescent clips from the outer serosal side of the colon and stomach. Then were used 532-nm diode laser and filter glass for visualizing the fluorescence signals through the colonic tissue. A 650-nm diode laser and a digital charge-coupled device (CCD) camera equipped with a band pass emission filter were used for the imaging of the fluorescent clips through the thick stomach tissue.[20] Nomikava used preoperative placement of endoscopic marking clips equipped with resin-conjugated fluorescent indocyanine green to determine the resection margin in eight patients with gastrointestinal cancer. During laparoscopic surgery, a dedicated laparoscopic system with a xenon light source was used to detect fluorescence. The evaluation determined whether the fluorescent from the clips was visualized during laparoscopic surgery.[21] Ryu et al evaluated the intraoperative visibility of the Zeoclip FS using a VISERA ELITE 2 and the short-term outcomes of 37 cancer patients who underwent preoperative fluorescent clip marking. Thirty-three patients exhibited clear fluorescent clip marking and easily determined transection lines. Fluorescence was not observed only in one sigmoid colon cancer patient, who required a colonic stent for preoperative obstruction. Three patients required additional procedures for fluorescence visualization. Kawaguchi used indocyanine green fluorescent imaging to detected the point of perforation in common bile duct. ICG-fluorescence imaging was used to detect grossly unidentifiable bile leak associated with a small perforation caused during the ERCP procedure. The intrabiliary route was selected for ICG administration because the ENBD tube had already been placed and intravenous injection could have decreased contrast between leaking bile and surrounding structures.[23]

Conclusion

In all of the studies mentioned, tattooing using this LED-activated fluorescence imaging technique and using novel fluorescently clips is a new concept for marking lesions and is useful, without any side effects, for identifying the location of a tumor in the perioperative period. The differential diagnosis of diseases of the oral mucosa should include the performance of other additional tests: bacteriological and mycological tests, viral cultures, allergy tests, serological tests, immunohistochemistry and immunofluorescence, as well as radiological tests (overview and intraoral radiography, panoramic dental tomography, radiovisiography, sialography), arthrography, angiography) and other imaging techniques such as: axial computed tomography, magnetic resonance, ultrasound and scintigraphy.

References

1. ECIS – European Cancer Information System. Colorectal cancer burden in EU-27. https://ecis.jrc.ec.europa.eu/pdf/Colorectal_cancer_factsheet-Mar_2021.pdf. Accessed: 07.06.2021.
2. Cunningham D, Atkin W, Lenz HJ, et al. Colorectal cancer. *Lancet*. 2010;375(9719):1030-1047.
3. Lin OS, Kozarek RA, Cha JM. Impact of sigmoidoscopy and colonoscopy on colorectal cancer incidence and mortality: an evidence-based review of published prospective and retrospective studies. *Intest Res.* 2014;12:268-274.
4. Kim S, Milsom J, Church J, et al. Perioperative tumor localization for laparoscopic colorectal surgery. *Surg Endosc.* 1997;11:1013-1016.
5. Feingold DL, Addona T, Forde KA, et al. Safety and reliability of tattooing colorectal neoplasms prior to laparoscopic resection. *J Gastrointest Surg.* 2004;8(5):543-546.
6. Sauntry JP, Knudtson KP. A technique for marking the mucosa of the gastrointestinal tract after polypectomy. *Cancer.* 1958;11:607-610.
7. Ponsky JL, King JF. Endoscopic marking of colonic lesions. *Gastrointest Endosc*. 1975;22:42-43.
8. Yang M, Pepe D, Schlachta CM, Alkhamesi NA. Endoscopic tattoo: the importance and need for standardised guidelines and protocol. *J R Soc Med*. 2017;110(7):287-291.
9. Shatz BA, Weinstock LB, Swanson PE, Thyssen EP. Long-term safety of India ink tattoos in the colon. *Gastrointest Endosc.* 1997;45(2):153-156.

10. Hyman N, Waye JD. Endoscopic four quadrant tattoo for the identification of colonic lesions at surgery. *Gastrointest Endosc.* 1991;37(1):56-58.
11. Fu KI, Fujii T, Kato S, Sano Y, Koba I, Mera K. A new endoscopic tattooing technique for identifying the location of colonic lesions during laparoscopic surgery: a comparison with the conventional technique. *Endoscopy.* 2001;33:687-669.
12. Nahid MAK, Shrestha AK, Imtiaz MR, Basnyat PS. Endoscopic tattooing for colorectal lesions: impact on quality of care and patient outcomes. *Ann Royal Coll Surg Engl*. 2020;102:8:594-597.
13. Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA. Indocyanine Green. Surgical Innovation. *Surg Innov*. 2016;23(2):166-175.
14. Ushimaru Y, Omori T, Fujiwara Y, et al. The Feasibility and Safety of Preoperative Fluorescence Marking with Indocyanine Green (ICG) in Laparoscopic Gastrectomy for Gastric Cancer. *J Gastrointest Surg*. 2019;23(3):468-476.
15. Tanaka C, Kanda M, Funasaka K, et al. Detection of indocyanine green fluorescence to determine tumor location during laparoscopic gastrectomy for gastric cancer: Results of a prospective study. *Asian J Endosc Surg.* 2020;13(2):160-167.
16. Nagata J, Fukunaga Y, Akiyoshi T, et al. Colonic marking with near-infrared, light-emitting, diode-activated indocyanine green for laparoscopic colorectal surgery. *Dis Colon Rectum*. 2016;59:e14-e18.
17. Ozawa Y, Murakami M, Watanabe M, et al. Preoperative colonic cancer tattooing using the near-infrared fluorescence laparoscopic imaging system. *Asian J Endosc Surg.* 2016;9(4):340-343.
18. Watanabe M, Tsunoda A, Narita K, Kusano M, Miwa M. Colonic tattooing using fluorescence imaging with light--emitting diode-activated indocyanine green: A feasibility study. *Surg Today.* 2009;39:214-218.
19. Barberio M, Pizzicannella M, Spota A, et al. Preoperative endoscopic marking of the gastrointestinal tract using fluorescence imaging: submucosal indocyanine green tattooing versus a novel fluorescent over-the-scope clip in a survival experimental study. *Surg Endosc.* 2020. doi: 10.1007/s00464-020-07999-2
20. Choi Y, Kim KG, Kim JK. A novel endoscopic fluorescent clip for the localization of gastrointestinal tumors. *Surg Endosc.* 2011;25:2372-2377.
21. Namikawa T, Iwabu J, Hashiba M, et al. Novel endoscopic marking clip equipped with resin-conjugated fluorescent indocyanine green during laparoscopic surgery for gastrointestinal cancer. *Langenbecks Arch Surg.* 2020;405(4):503-508.
22. Ryu S, Okamoto A, Nakashima K, et al. Usefulness of Preoperative Endoscopic Fluorescent Clip Marking in Laparoscopic Gastrointestinal Surgery. *Anticancer Res.* 2020;20(11):6517-6523.
23. Kawaguchi Y, Ishizawa T, Harada Y, et al. Intraoperative Identification of Bile Duct Perforation Following ERCP Using Indocyanine Green-Fluorescence Imaging. *Dig Dis Sci.* 2014;59:1063-1065.

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REVIEW PAPER

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Herbarium – Summer 2021

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ABSTRACT

Introduction. Medicinal plants are perfectly suited to interact with biological macromolecules like proteins and nucleic acids. All sources of natural products such as plants, microorganisms, animals, etc. are therefore biochemical potential.

Aim. The goal was to present a typical plant often found in abandoned places, fields, meadows in the Podkarpacie region of Poland. Medicinal plants presented here were collected in Summer 2021 close to the city Jarosław.

Material and methods. Plants collected for herbarium, immediately after harvest, are laid out between paper sheets that absorb moisture. Dried plants retain the shape of individual organs and usually also the colors. As the result of our collection, in this article are presented *Papaver rhoeas* L., *Centaurea cyanus* L., *Capsella bursa – pastoris* L., *Taraxacum officinalle* F.H., Wigg Coll and *Lamium album* L.

Analysis of the literature. The desire to summarize information for future generations and to present the writings of the classical scholars to a wide audience was the major stimulations for presenting a most popular medicinal plants to the reader. The plants most often obtained for pharmaceutical purposes include: chamomile, fennel, St. John's wort, nettle, mint, dandelion, yarrow, marshmallow, sage, foxglove, lemon balm, dandelion, rosehip, aloe, ginseng, and milk thistle.

Conclusion. The history of plants collections is well documented.

Keywords. biochemistry, herbarium, medicinal plants

Papaver rhoeas L.

Family: *Papaveraceous* - poppy family

Medicinal Raw Material: *Flos Rhoeados* - field poppy flower (Fig. 1)

Active compounds: For the active compounds of poppy field include alkaloids (mainly readina), isoquinoline alkaloids, anthocyanins, acids organic, phytosterol, mucus compounds and salts mineral.

Pharmacological action: It has a mild effect calming, analgesic and expectorant. It is used for catarrhs of the mouth, like also for sore throat, hoarseness and troublesome cough. The field poppy also has properties anti-inflammatory and regenerating, that's why it is used as an aid in inflammation of the mucous membranes and skin. Poppy seed decoctions are used to rinse the throat and cavity orally to eliminate irritation.[1-14]

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Fig. 1. *Papaver rhoeas*, with common names including common poppy, corn poppy, corn rose, field poppy, Flanders poppy, and red poppy, is an annual herbaceous species of flowering plant in the poppy family *Papaveraceous*

Centaurea cyanus L.

Family: *Asteraceae* (Compositae) - Asteraceae (complex)

Name of the medicinal raw material: Flos Cyani - cornflower flower (Fig. 2)

Active compounds: Until the active compounds present the flowers of cornflower blueberry include: cyanine, pelargonidine, copigment (flavone glycoside Apigenin 4ß-D-glucose-7ß-D-glucoronide), centaurin, sesquiterpene lactones and minerals, among which manganese is very important.

Pharmacological action: In cosmetology cornflower flower extract was used, which is a moisturizing ingredient. Additionally, this extract is also potent strengthening as well as calming. The centaurin contained in the flowers exhibits anti-radical, soothing and anti-inflammatory properties. Aqueous cornflower flower extract is used to make eye drops - decongestant, anti-irritation. Cornflower flowers are also included often used in digestive and soothing mixtures gastric disorders as well as diuretics. Are also an additive to creams and ointments used for brittleness blood vessels.[1-14]

Fig. 2. *Centaurea* is a genus of between 350 and 600 species of herbaceous thistle-like flowering plants in the family *Asteraceae*

Capsella bursa-pastoris L.

Family: *Brassicaceae - cruciferous*

Name of the medicinal raw material: *Herba Bursae pastoris* - grass of the grass cutter (Fig. 3)

Active compounds: Amines are present in the grass of the grasshopper biogenic (choline, acetylcholine, tyramine, histamine), aliphatic acids (fumaric acid), aromatic acids (chlorogenic, syringic, vanilla, coumarin), flavonoids (mainly diosmin and rutin), phytosterols, vitamins: A, K and C, potassium and calcium and essential oils.

Pharmacological action: *Capsella bursa-pastoris* herb has a strong anti-inflammatory effect, supporting the work of the heart muscle and uterus and properties anti-cancer. Thanks to the routine contained in the grass, the raw material this improves the functioning of the circulatory system, and women use it in to relieve menstrual discomfort. *Capsella bursa-pastoris* also has a soothing effect on the urinary system, which is why it is used in inflammation of the gland the prostate, bladder and kidneys.[1-14]

Fig. 3. *Capsella bursa-pastoris L.*

Fig. 4. *Taraxacum officinalle* F.H. Wigg. Coll

Taraxacum officinalle F.H. Wigg. Coll

Family: *Asteraceae* (Compositae) – *Asteraceae* (folded)
Name of the medicinal raw material: *Taraxaci officinalis radix* – dandelion root (FP XI) *Taraxaci officinalis herba cum radice* – herb nun with root (FP XI) (Fig. 4)
Active compounds: Until the active compounds present in the dandelion root there are lactones sesquiterpene. However, milk juice contains triterpene alcohols, and flowers – 16-hydroxy derivatives of taraxasterols, β-sitosterol, stigmasterol and campesterol. Very important active compounds the leaves contain flavonoids (derivatives of, among others, luteolin and quercetin). The substances that are present in the whole plant are polyphenolic acids (acid chicory, chlorogenic, caftar, coffee). The dandelion contains also numerous carotenoids, coumarins, carbohydrates, waxes and choline.
Pharmacological action: This raw material acts as a gentle agent choleretic and diuretic. Due to the numerous substances turpentine, with a bitter taste, perfectly stimulates the appetite. In many countries, including France, fresh young leaves are recommended as salad for the so-called spring treatments. The root of the common dandelion is also used for supportive treatment in diseases liver, gallbladder and digestive disorders. Moreover, *Taraxacum officinale* has been reported to possess antioxidant activities. Due to the content of flavonoids, this plant also has potential anti-glycation properties.[1-16]

Lamium album L.

Family: *Lamiaceae (Labiatae) – Lamiaceae (labial)*
Medicinal Raw Material: *Lamii albi flos* – the flower of light *Lamii albi herba* – herb of luminosity (Fig. 5)
Active compounds: White jasmine contains compounds active substances such as flavonoids (rutin and quercetin), organic acids (acid cinnamon, rosemary, p-coumarin, chlorogenic, caffeoylquinic acid), numerous iridoid glycosides, tannins, saponins, mucus compounds and essential oils. Herb white light additionally contains vitamin C and provitamin A.
Pharmacological action: White light herb found wide anti-hemorrhagic, astringent, anti-inflammatory, choleretic use, diastolic, antibacterial and facilitating digestion. Thanks to these teas containing white light herb help with plenty menstruation and have a positive effect on the functioning of the system digestive system – support the work of the liver and pancreas. In medicine white light rinses are also used to facilitate healing wounds in the mouth and help with pharyngitis and laryngitis. In addition, jasmine is also added to facilitating syrups expectoration. It is also used in wall strengthening agents.[1-14]

Fig. 5. *Lamium album* L.

Conclusion

In this album the pharmaceutical most important families were presented and highlighted. Since these species are mostly known as a botanical medicinal plants.

References

1. Matthias A, Laisné N. *Medicinal Plants: Production, Cultivation and Uses.* Nova Scientific NY, USA. 2017. ISBN: 978-1-53612-728-7.
2. Bartusik-Aebisher D, Aebisher D. (Editor's). *Biochemical Guide to Medicinal Plants.* Nova Scientific NY, USA. 2020. ISBN: 978-1-53618-902-5.
3. Govil JN, Tiwari L (Editor's). *Recent Progress in Medicinal Plants, Phytoconstituents and Biochemical Processes.* Studium Press (INDIA) PVT.LTD. 2013. ISBN: 9781933699257.
4. Allkin B. *Useful Plants – Medicines: At Least 28,187 Plant Species are Currently Recorded as Being of Medicinal Use.* In: Willis KJ, editor. State of the World's Plants 2017. London (UK): Royal Botanic Gardens, Kew; 2017.
5. Paton A, Allkin R, Belyaeva I, et al. *Plant name resources: building bridges with users.* In: Rakotoarisoa NR, Blackmore S, Riera B, editors. Botanists of the twenty-first century: roles, challenges and opportunities. Paris (France): United Nations Educational, Scientific and Cultural Organisation (UNESCO); 2016.
6. Allkin B. *Communicating safely & effectively using plant names.* In: Mukerjee PK, editor. Traditional Medicines and Globalisation: The Future of Ancient Systems of Medicine. Kolkata (India): Maven Publishers; 2014.
7. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *IARC Monogr Eval Carcinog Risks Hum.* 2002;82:1-556.
8. Hardy ML, Coulter I, Venuturupalli S, et al. Ayurvedic interventions for diabetes mellitus: a systematic review. Evid Rep Technol Assess (Summ). 2001;(41):2p.
9. Mulrow C, Lawrence V, Ackermann R, et al. Garlic: effects on cardiovascular risks and disease, protective effects against cancer, and clinical adverse effects. *Evid Rep Technol Assess (Summ).* 2000;(20):1-4.
10. Mulrow C, Lawrence V, Jacobs B, et al. *Milk thistle: effects on liver disease and cirrhosis and clinical adverse effects.* Evid Rep Technol Assess (Summ). 2000;(21):1-3.
11. Sen T, Samanta SK. Medicinal plants, human health and biodiversity: a broad review. *Adv Biochem Eng Biotechnol.* 2015;147:59-110.
12. Banerjee J, Das A, Sinha M, Saha S. Biological Efficacy of Medicinal Plant Extracts in Preventing Oxidative Damage. *Oxid Med Cell Longev.* 2018;2018:7904349.
13. Chandran R, Abrahamse H. Identifying Plant-Based Natural Medicine against Oxidative Stress and Neurodegenerative Disorders. *Oxid Med Cell Longev.* 2020;2020:8648742.
14. Domka W, Aebisher D, Truszkiewicz A, Bartusik-Aebisher D. Medicinal & Aromatic Plants. *Eur J Clin Exp Med.* 2018;16(4):350-358.
15. Huang S, Meng N, Liu Z, Guo L, Dong L, Li B, Ye Q. Neuroprotective Effects of Taraxacum officinale Wigg. Extract on Glutamate-Induced Oxidative Stress in HT22 Cells via HO-1/Nrf2 Pathways. Nutrients. 2018;10(7):926.
16. Sadowska-Bartosz I, Galiniak S, Bartosz G. Polyphenols protect against protein glycoxidation. *Free Radic Biol Med.* 2014;75 Suppl 1:S47.

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CASUISTIC PAPER

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Pericallosal berry aneurysm associated with azygous anterior cerebral artery – a case report

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ABSTRACT

Introduction. Azygos anterior cerebral artery is a rare variant of the anterior segment of the circle of Willis caused by an unusual fusion of the normally paired A2 segments of the anterior cerebral artery (ACA). Despite its rare occurrence, it is associated with various vascular and structural cerebral abnormalities, particularly berry aneurysms.

Aim. We aimed to present a case of a 41-year-old female patient who presented to our neurology department with complaints of headache.

Description of the case. She had a positive paternal history of aneurysmal subarachnoid hemorrhage. Magnetic resonance angiography (MRA) of her brain revealed an azygos ACA (bifurcating into two pericallosal arteries) which was associated with a saccular aneurysm at its bifurcation point. She was referred to the interventional radiology department for preventive endovascular treatment.

Conclusion. Azygos ACA carries a high risk of aneurysm development and its occlusion can potentially compromise blood supply to both cerebral hemispheres. It is therefore crucial for clinicians to be aware of its significance and to report its presence in angiographic studies.

Keywords. azygos ACA, berry aneurysm, magnetic resonance angiography

Introduction

Azygos anterior cerebral artery (ACA) is a variant of the anterior segment of the circle of Willis characterized by a single A2 segment, in which both ACA territories are supplied by this single A2 trunk. The underlying pathological mechanism is the persistence of embryonic median artery of the corpus callosum.[1] It was first described by Wilders, as arteria termatica.[2] It is a rare variation of the ACA with an incidence of about 0.4-1% in the general population.[3] There are different branching patterns of this variation. More frequently it divides into two pericallosal arteries, but it may also trifurcate into two pericallosal arteries and one callosomarginal artery or quadrifurcate into two pericallosal arteries and two callosomarginal arteries.[4,5] Despite its rare occurrence, it is associated with various vascular and structural cerebral abnormalities, particularly berry aneurysms.

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Aim

We aimed to present a case of an azygos ACA bifurcating after a segment of a common trunk and having an aneurysm at this bifurcation point.

Description of the case

A 41-year-old female patient presented to our neurology department with complaints of headache of increasing frequency and severity. She had a positive paternal history of aneurysmal subarachnoid hemorrhage. On magnetic resonance angiography (MRA) of the brain, an azygos ACA bifurcating into two pericallosal arteries after a 7 mm segment of the fused azygos trunk was established. There was a 4x3.7 mm sized saccular aneurysm projecting antero-superiorly at this bifurcation point (Figure 1). There was no ACoA as expected, but the rest of the circle of Willis was unremarkable. She was referred to the interventional radiology department for preventive endovascular treatment.

Discussion

Azygos ACA is one of the clinically important anomalies of the circle of Willis, because its occlusion affects both hemispheres. On the other hand, cerebral aneurysms are frequent with a reported incidence varying between 13 to 71% (2), which is very high when compared with the reported incidence of cerebral aneurysms (0.2-8.9) in the unaffected general population.[6] In a review of 36 cases of distal ACA aneurysms, 6 cases (17%) were associated with azygos ACA.[7] In another study including 105 patients with subarachnoid hemorrhage, among 8 cases of distal ACA aneurysms, 2 (1.9%) cases were associated with azygos ACA with the incidence rate of 25%.[8] The typical location for aneurysm development is at the bifurcation point of the common trunk, likely due to alterations in the hemodynamics at this site. Congenital abnormalities in the vessel walls may also be contributory to the development of azygos ACA aneurysms.

Although the rest of the circle of Willis was unremarkable in our patient, other vascular anomalies or variations may also be associated with azygos ACA. These include absence of the anterior communicating artery (ACoA) as in our case. In a retrospective study by Beyhan et al. including a total of 4913 cases who had brain computed tomography angiography (CTA), MRA, contrast-enhanced magnetic resonance imaging (MRI) and digital substraction angiography (DSA), azygos ACA was observed in 57 cases with the frequency of 1.16 %. In this study, unilateral vertebral artery hypoplasia and ACA A1 segment hypoplasia were the most common associated vascular variations.[9] Although not present in our patient, as a midline vascular variation, azygos ACA is more frequent in patients with various other midline abnormalities including dysgenesis of corpus callosum, lobar holoprosencephaly or septo-optic dysplasia.[10]

Conclusion

Azygos ACA is a rare but clinically important anomaly of the circle of Willis, with a high risk of aneurysm development which can sometimes be established incidentally on neuroimaging studies. It is noteworthy that its occlusion can potentially compromise blood supply to both cerebral hemispheres. Therefore, it is crucial for clinicians to be aware of its significance and to report its presence in angiographic studies.

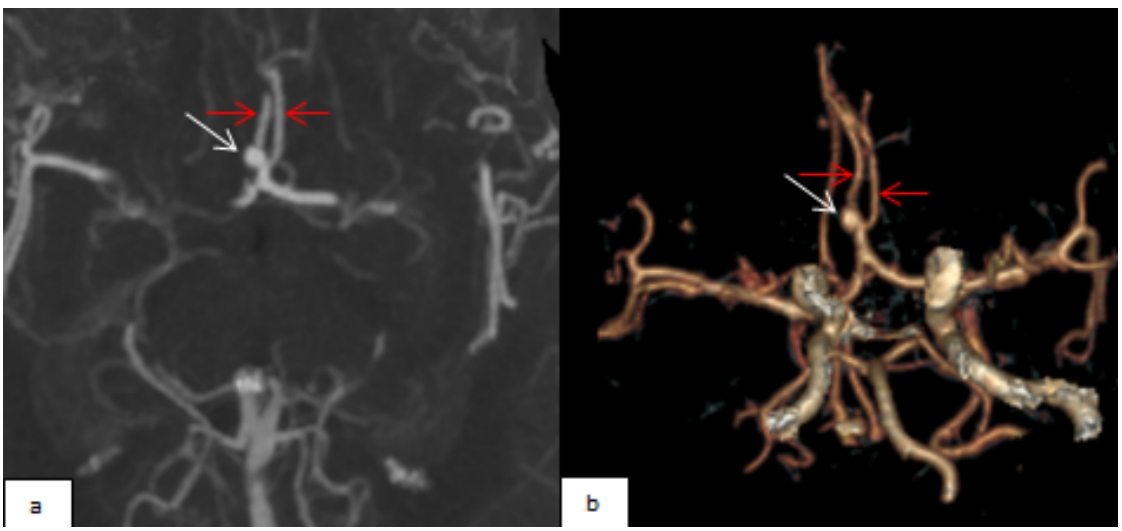

Fig. 1. Coronal maximum intensity projection image (A) and three-dimensional reformatted image (B) of contrast-enhanced MRA showing a saccular aneurysm projecting antero- superiorly (white arrows) from the bifurcation point of the azygous ACA. Two pericallosal arteries are shown (red arrows). There is no ACoA

References

1. Dimmick SJ, Faulder KC. Normal variants of the cerebral circulation at multidetector CT angiography. *Radiographics.* 2009;29(4):1027-1043.
2. Schick RM, Rumbaugh CL. Saccular aneurysm of the azygos anterior cerebral artery. *AJNR Am J Neuroradiol.* 1989;10(5):73.
3. Huh JS, Park SK, Shin JJ, et al. Saccular aneurysm of the azygos anterior cerebral artery: three case reports. *J Korean Neurosurg Soc.* 2007;42(4):342-345.
4. Kaspera W, Ładziński P, Słowiński J, et al. Blood flow velocity in the arteries of the anterior cerebral artery complex in patients with an azygos anterior cerebral artery aneurysm: a transcranial color-coded sonography study. *Clin Neurol Neurosurg.* 2009;111(1):63-68.
5. Singh H, Dhandapani S, Mathuriya SN. Types of Azygos Distal Anterior Cerebral Artery Branching Patterns: Relevance in Aneurysmal Surgery. *Cureus.* 2016;8(7):e681.
6. Takahashi S. *Neurovascular Imaging, MRI & Microangiography*. Springer Verlag. 2010.
7. Katz RW, Horoupian DS, Zingesser L. Aneurysm of azygous anterior cerebral artery. A case report. *J Neurosurg.* 1978;48(5):804-808.
8. Ghanta RK, Kesanakurthy MV, Vemuri VN. Aneurysm of azygos anterior cerebral artery: A report of two cases. *Asian J Neurosurg.* 2016;11(3):312.
9. Beyhan M, Gökçe E, Karakuş K. Radiological classification of azygos anterior cerebral artery and evaluation of the accompanying vascular anomalies. *Surg Radiol Anat.* 2020;42(11):1345-1354.
10. Szdzuy D, Lehmann R, Nickel B. Common trunk of the anterior cerebral arteries. *Neuroradiology.* 1972;4(1):51-56.

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CASUISTIC PAPER

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Embolization as a method of treating aneurysms intracranial – a case study

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ABSTRACT

Introduction. Cerebrovascular accidents are the third leading cause of death in developed countries. It is a very debilitating disease of the brain structures in which aneurysms are located. For a long time, the only method of treating intracranial aneurysms was surgery. However, mainly due to the difficult placement of the aneurysms, surgical treatment was very difficult. Therefore, in 1953, the method of percutaneous arterial catheterization was developed, which became the basis for the improvement of the field of interventional radiology.

Aim. The aim of the research is to describe the method of embolization of intracranial aneurysms and to illustrate it in selected clinical cases.

Description of the case. Surgical management of a 60-year-old woman with intracranial aneurysm of the anterior communicating artery after performing computed tomography with contrast.

Conclusion. The method of embolization of intracranial aneurysms is a reliable and accurate surgical method that significantly improves the health condition, quality of life of patients and reduces the mortality rate of cerebrovascular events.

Keywords. embolization, intracranial aneurysms, neurosurgical procedures in the treatment of aneurysms

Introduction

Intracranial aneurysm is one of the most common diseases of the neurovascular system. They are called weakened vasodilations and belong to the group of cerebrovascular accidents. They are the third leading cause of death in developed countries. Intracranial aneurysms is the disease that significantly destroys the structures of the brain in which it is located. Cerebrovascular accidents have a complex but not fully understood etiology.[1] Researchers from the University of Portugal have shown that intracranial aneurysms are the most common type of malignant tumors observed at the clinical stage due to a specific mechanism of its development and occurrence.[2]

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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The aneurysm ruptures in more than 50% of cases, which results in spontaneous subarachnoid haemorrhage (SAH) in approximately 80% of patients.[3] The most catastrophic consequence of aneurysm rupture is 50% mortality and in 30-50% of cases the cause of severe neurological complications resulting in significant disability in survivors.[4]

There are numerous scientific reports in foreign literature that describe the pathogenesis of aneurysms. These cerebrovascular events happen as a result of arterial dilatation due to acquired or congenital lesions leading to a decrease in the mechanical resistance of the artery walls. Most often it is a type of damage to the structure of the vessel wall with a characteristic lack or weakening of the muscular and/or elastic membrane, which may cause the inner membrane to bulge outside the vessel.[5-7] In the last few decades, surgery has been the only treatment for intracranial aneurysms. However, the neurosurgical treatment was very limited by the difficult location of the aneurysms, making surgery impossible. Hence, in 1953, the method of percutaneous arterial catheterization was described by the Swedish radiologist Sven Seldinger, which became the foundation for the development of a new field – interventional radiology.[8-10]

The embolization procedure consists in placing platinum, detachable embolization coils in the aneurysm sac in order to fill it as much as possible, so that the aneurysm is excluded from the cerebral circulation. The spirals are placed through a microcatheter, the end of which is in the aneurysm sac. Most often, the procedure is performed through access from the common femoral artery. In rare situations, e.g. in the case of obstruction of both common iliac arteries, it can be performed through the brachial artery or directly through the common carotid artery.

Currently, it is found that the embolization method can disable as much as 80% of all existing aneurysms by placing embolization materials into the vessel lumen using a microcatheter. Patients' quality of life has improved since intracranial embolization began, and the mortality rate associated with such cerebrovascular incidents has significantly decreased.

Aim

The aim of this study is to describe the method of embolization of intracranial aneurysms and to illustrate it in a selected clinical case, treated at the St. Queen Jadwiga Clinical District Hospital, No 2 in Rzeszow. In order to explore the topic, scientific articles, medical books and databases were analyzed in detail, i.e. PubMed, ScienceDirect, Google Scholar, EBSCO and Free Medical Journals.

Description of the case

A 60-year-old patient came to the Hospital Emergency Department (HED), claiming that the cause of her health was a two-day constant headache, nausea, general malaise and vision problems. Neurological symptoms were not found. The patient reported a possible fall at home as the cause of the headache. The doctor on duty referred the patient to a computed tomography with contrast, which showed the aneurysm on the anterior connecting artery. The woman was admitted to the Department of Neurosurgery, where a neurosurgeon qualified her for cerebral angiography. The examination was performed using the Seldinger method of puncture of the right common femoral artery. Both common carotid arteries and the left vertebral artery were catheterized. The right internal carotid artery was additionally examined by rotational angiography and spatial reconstructions were performed. Visipaque non-ionic contrast agent was given. In the area of the anterior connecting artery, a sac-like aneurysm with dimensions of 4.5x3x2.7 mm, filled from the right side, was visible. Some traces of contrast agent from the left side were also found. The aneurysm is directed upwards and to the left side, the neck of the aneurysm is 2.1x1.7 mm. Funnel-shaped exit of the right posterior connecting artery. Hypoplastic P1 segment of the left posterior artery of the left brain, with visible supply of the posterior brain artery through the posterior connecting arteries (carotization). The examination showed the correct image of the main arterial trunks and their branches of deep veins and superficial brain and dural sinuses. Other cerebral vascular malformations were not visualized. The above description is illustrated in figures 1, 2, 3.

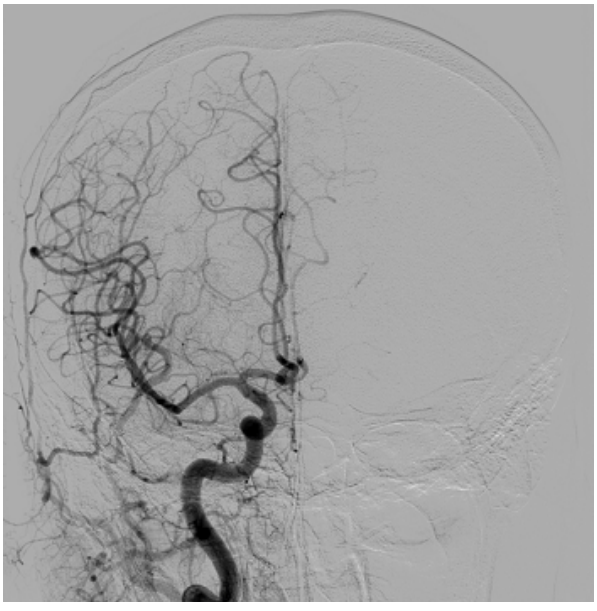

Fig. 1. Cerebral angiography in the AP projection (the arrow in the figure shows the aneurysms of the anterior communicating artery, own source)

Description of the aneurysm embolization procedure

The patient received general anesthesia. From the puncture of the right common femoral artery, the right in-

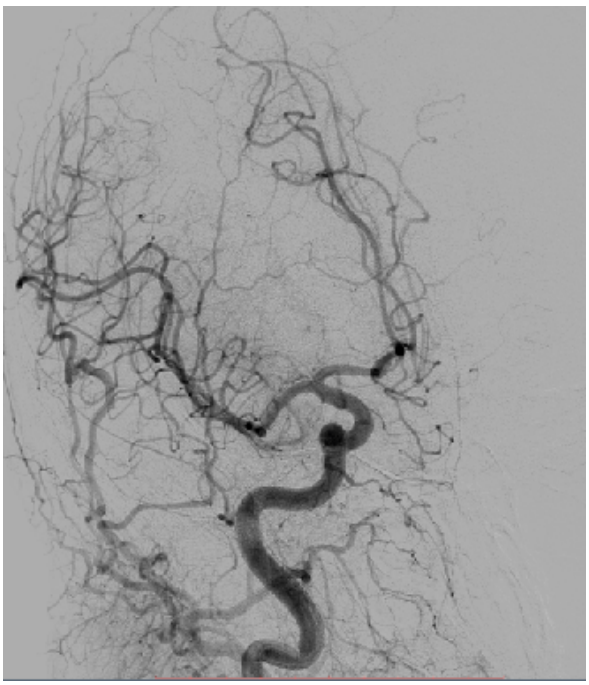

Fig. 2. Cerebral angiography in oblique projection (the arrow in the figure shows the aneurysms of the anterior communicating artery, own source)

Fig. 3. Cerebral angiography study in 3D reconstruction (dimensions described above, own source)

ternal carotid artery was catheterized by the Seldinger method and angiography was performed which showed a sac-like aneurysm on the anterior communicating artery. Two embolization coils were inserted into the aneurysm sac using a microcatheter. Control angiographs showed complete closure of the aneurysm, and the surrounding vessels had patency. The course of the procedure without complications. The puncture site of the right common femoral artery was closed with the Angio-

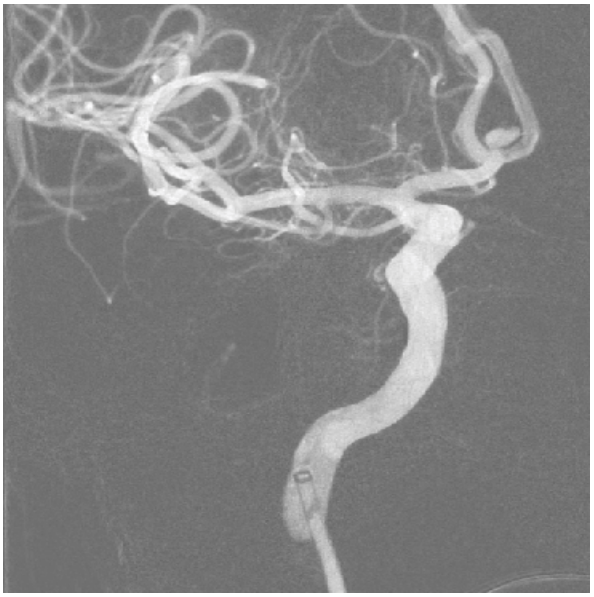

Fig. 4. Beginning of the procedure of the anterior communicating artery aneurysm (own source)

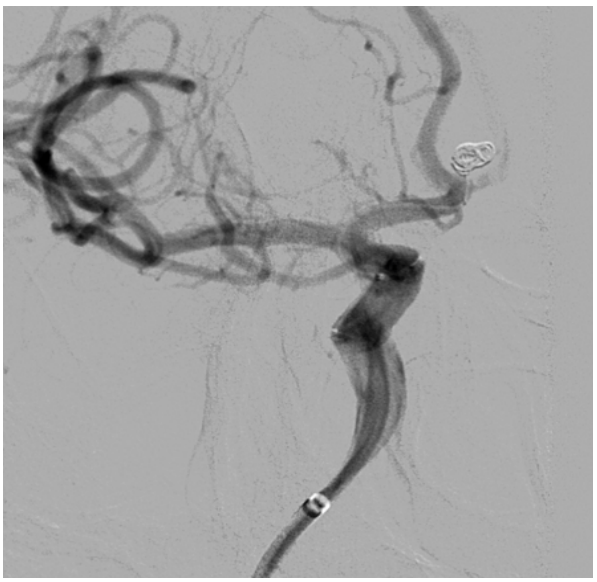

Fig. 5. Procedure of the anterior communicating artery aneurysm (own source)

Seal system. The material from which the embolization coils were made is not a contraindication to placing the patient in the magnetic field during the magnetic resonance imaging (Fig. 4-7).

Discussion

Cerebrovascular accidents are the third leading cause of death in developed countries. This group of cerebrovascular incidents includes, inter alia, intracranial aneurysms, i.e. pathological dilatation of cerebral vessels occurring at bifurcations of intracranial arteries. It is a very debilitating disease of the brain structures in which aneurysms are located. It is estimated that intra-

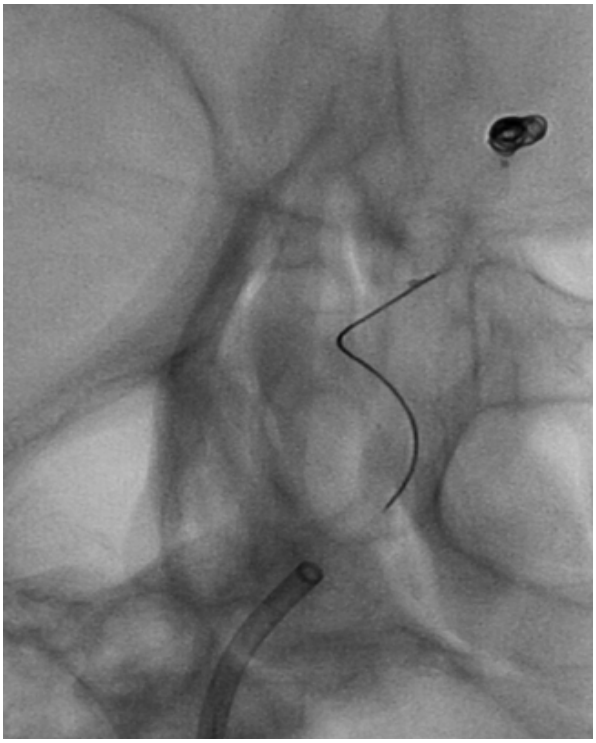

Fig. 6. Procedure of the anterior communicating artery aneurysm (no contrast, own source)

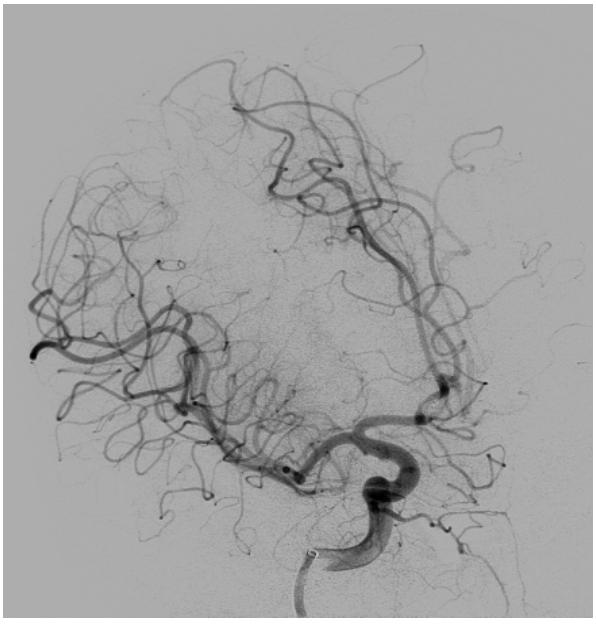

Fig. 7. The end of the aneurysm embolization procedure (own source)

cranial aneurysms occur in 6% of the world's population, of which 2% develop aneurysm rupture due to the incidence of subarachnoid haemorrhage.[11] Non-ruptured intracranial aneurysms can be diagnosed accidentally by imaging the skull to detect damage/injuries or other pathologies, or by targeted high-risk screening, i.e .: in people with a family history of intracranial aneurysms.[12] The most destructive consequence of an intracranial aneurysm rupture is subarachnoid haemorrhage, which may be mildly symptomatic or lead to severe disability or even death. It is estimated that the mortality associated with subarachnoid haemorrhage affects approximately ⅓ to ½ of all patients.[12-13] However, despite its prevalence and impact on the body, the etiopathogenesis of intracranial aneurysms is still unknown.[14] In 85% of patients, intracranial aneurysms are located on the anterior part of the Willis arterial circle, the most of which, i.e. 35%, can be located on the anterior connecting artery.[15] According to the scientific research conducted by Juvel, it can be concluded that the risk of bleeding aneurysm located in front of the arterial circle of the brain ranges from 1.6-1.9% per year.[16]

In the case described above, the physical examination revealed the following symptoms: 2-day severe headache, nausea, general malaise and vision problems. The symptoms described by the patient probably appeared after the fall at home. The symptoms mentioned by the patient may indicate many injuries within the brain and head, hence the doctor on duty referred the patient for further imaging tests. Computed tomography of the head was performed with contrast, which showed an aneurysm of the anterior connecting artery. After the medical history and physical examination, the patient was admitted to the Department of Neurosurgery, where a neurosurgeon qualified her for cerebral (diagnostic) angiography.

The decision to choose the embolization treatment of intracranial aneurysms was made immediately after the diagnostic angiography was performed. In the studies by Petridis et al., the symptoms of the patient of the Provincial Clinical Hospital No. 2 named after St. Jadwiga Queen in Rzeszow are characteristic symptoms of intracranial aneurysm.[17] Computed tomography is a very sensitive examination (about 95%) and can reveal deficits within the examined area.[17] Computed tomography with contrast ensures the visualization of the cerebral vessel in three dimensions, which enables a precise assessment of vascular brain and the base of the skull bone, making it much easier to plan the surgical procedure.[18]

After the surgery, the patient was carefully looked after and monitored. As indicated, the patient was given prophylactic cerebral-protective, analgesic, antibacterial and sedative agents. At the discharge from the hospital, the patient was informed about the necessity to attend a follow-up visit, during which diagnostic angiography would be performed again to detect recurrence of the intracranial aneurysm. The test result was negative.

Intracranial aneurysms are now detected more frequently due to improved imaging techniques. After detecting a lesion, the natural history of the aneurysm should be compared with the risk of planned surgery, taking into account the size, morphology, location of the aneurysm, as well as the patient's age and comorbidities. The patient's history and physical examination should also be

very scrupulous.[1] The endovascular embolization treatment of aneurysms has become an acceptable treatment with good clinical outcomes and provides adequate protection against re-bleeding and rupture of aneurysms.[19]

Intracranial aneurysms are among the most common cerebrovascular defects. These defects are the third leading cause of death in the world in developed countries. By definition, intracranial aneurysms are cerebrovascular events arising from the weakening of blood vessels within the damaged area. The disease significantly destroys the brain structures in which aneurysms are located, contributing to long-term disability or death. The etiology of cerebrovascular defects is still not fully understood.

In recent decades, the only method of treating intracranial aneurysms has been surgery, but due to the limited possibilities of imaging tests and the difficult location of cerebrovascular lesions, treatment has been significantly limited or even impossible. Hence, in the 1950s, the method of percutaneous arterial catheterization – the Seldinger method – was described and presented. The innovative technique has become the basis for the development of interventional radiology. Thanks to the Swedish radiologist (Sven Seldinger), the diagnosis of intracranial artery aneurysms has increased significantly, which resulted in an increase in the number of procedures performed and the recognition of cerebrovascular incidents has increased.

Currently, it is determined that the embolization method can disable up to 80% of all existing aneurysms by placing embolization materials into the vessel lumen using a microcatheter.

Since intracranial embolization procedures have begun, patients' quality of life has improved, the mortality rate has decreased, and the level of disability associated with such cerebrovascular events has decreased.

Conclusion

As a result of the analysis of the literature and own research, the following conclusion was drawn, i.e. treatment of intracranial artery aneurysms using the embolization method – the Seldinger method is a fundamental treatment that results in positive effects, and additionally reduces the level of disability and death caused by cerebrovascular defects.

References

1. Yon KI, Shang HS, Seung KB. Complications of endovascular treatment for intracranial aneurysms: Management and prevention. *Interv Neuroradiol*. 2018;24(3):237-245.
2. Campodeaño L, Oliveira MSN., Pinho FT. A review of computational hemodynamics in middle cerebral aneurysms and rheological models for blood flow. *Appl Mech Rev*. 2015;67(3):030801.
3. Amenta PS, Dalyai RT, Kung D, et al. Stent-assisted coiling of wide-necked aneurysms in the setting of acute subarachnoid hemorrhage: experience in 65 patients. *Neurosurgery*. 2012;70(6):1415-1429.
4. Texakalidis P, Sweid A, Mouchtouris N, et al. Aneurysm Formation, Growth, and Rupture: The Biology and Physics of Cerebral Aneurysms. *World Neurosurg*. 2019;130:277-284.
5. Avery RK, Albadawi H, Akbari M, et al. An injectable shear-thinning biomaterial for endovascular embolization. *Sci Transl Med*. 2016;16;8(365):365ra156.
6. Calero A, Illig KA. Overview of aortic aneurysm management in the endovascular era. *Semin Vasc Surg*. 2016;29(1-2):3-17.
7. Mocco J, Brown RD, Torner JC, et al. International Study of Unruptured Intracranial Aneurysms Investigators. Aneurysm Morphology and Prediction of Rupture: An International Study of Unruptured Intracranial Aneurysms Analysis. *Neurosurgery.* 2018;1;82(4):491-496.
8. Vivancos J, Gilo F, Frutos R, et al. Clinical management guidelines for subarachnoid haemorrhage. Diagnosis and treatment. *Neurologia.* 2014;29(6):353-370.
9. Gabriel J, Rinkel E. Management of patients with aneurysmal subarachnoid haemorrhage. *Curr Opin Neurol*. 2016;29(1):37-41.
10. Gralak-Łachowska D, Lewandowski P. Dostępy naczyniowe w kardiologii inwazyjnej. *Postępy Nauk Medycznych* 2015;28(11B):74-78.
11. 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.
12. Nieuwkamp DJ, Setz LE, Algra , et al. Changes in case fatality of aneurysmal subarachnoid haemorrhage over time, according to age, sex, and region: a meta-analysis. *Lancet Neurol.* 2009;8:635-642.
13. Greving JP, Wermer MJ, Brown RD, et al. Development of the PHASES score for prediction of risk of rupture of intracranial aneurysms: a pooled analysis of six prospective cohort studies. *Lancet Neurol.* 2014;13:59-66.
14. Vlak MH, Algra A, Brandenburg, et al.Prevalence of unruptured intracranial aneurysms, with emphasis on sex, age, comorbidity, country, and time period: a systematic review and meta-analysis. *Lancet Neurol.* 2011;10:626-636.
15. Ząbek M. *Krwawienie podpajęczynówkowe. W: Zarys neurochirurgii*. Red. Ząbek M. Warszawa: PZWL; 1999.
16. Juvela S. Growth and rupture of unruptured intracranial aneurysms. *J Neurosurgery* 2019;131:843-851.
17. Petridis AK , Kamp MA, Cornelius JF, et al. Aneurysmal Subarachnoid Hemorrhage. *Dtsch Arztebl Int*. 2017;31;114(13):226-236.
18. Ajiboye N, Chalouhi N, Starke MR i wsp. Unruptured Cerebral Aneurysms: Evaluation and Management. *Scientific World Journal.* 2015;2015:954954.
19. Wan J, Gu W, Zhang X, et al. Endovascular coil embolization of aneurysm neck for the treatment of ruptured intracranial aneurysm with bleb formation. *Med Sci Monit*. 2014;20:1121-1128.

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CASUISTIC PAPER

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Basal ganglia calcifications is not inconsequential in pediatric cases

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ABSTRACT

Introduction. Basal ganglia calcification (BGC) in pediatric population is rare and is considered as a pathological finding. Various causes may be responsible for BGC including hypoparathyroidism, various infectious, toxicities or hereditary disorders.
Aim. We aimed to present a 8 year old boy presented with generalized seizure and bilateral small amount of globus pallidum calcifications on neuroimaging studies leading to the diagnosis of idiopathic hypoparathyroidism, which is a treatable cause of seizure.
Description of the case. A 8-year-old boy presented to our emergency department with generalized seizure for the first time in his life. There was no history of previous head trauma and his family history was unremarkable. Neurological examination revealed no pathological findings. Radiological imaging studies revealed only bilateral small amount of globus pallidus calcifications. He was referred to the pediatric endocrinology department for further evaluation of the hypocalcemic convulsion, where laboratory investigations revealed idiopathic hypoparathyroidism as the cause of hypocalcemic convulsion with exclusion of the other causes.
Conclusion. Even a small amount of BGC in pediatric patients may be the sign of primary hypoparathyroidism and should be evaluated with serum electrolyte levels for early diagnosis and for the prevention of multisystemic complications of hypoparathyroidism.
Keywords. basal ganglia calcification, idiopathic hypoparathyroidism, seizure

Introduction

Isolated basal ganglia calcifications (BGCs) on neuroimaging studies particularly on head computed tomography (CT) is a frequently observed phenomenon in elderly patients and is generally considered as an idiopathic age related incidental finding. Lentiform nucleus particularly globus pallidus is the most common location for these calcifications.[1] In addition to aging, idiopathic BGCs may also be primary familial in origin, which is known as Fahr disease. BGC in these cases typically presents in ages between 40 and 60.[2] However, recently it has been shown that it can also be observed in pediatric cases with chromosomal deletions on 8p11.[3] However, in pediatric patients, it is more common that BGCs occur as a result of secondary causes, including parathyroid disorders (hypoparathyroidism, pseudo-

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hypoparathyroidism), nephrotic syndrome, infections (like TORCH, brucellosis), various congenital disorders (like Cockayne syndrome), radiation or chemotherapy, toxicities (like hypervitaminosis D), which are all known as Fahr syndrome.[2,4-6] Nevertheless, BGC in pediatric population is rarer than adults and is considered as a pathological finding.

Aim

We aimed to show the importance of BGCs detected on pediatric patients and the necessity for further evaluation.

Description of the case

A 8-year-old boy presented to our emergency department with generalized seizure for the first time in his life. His mother defined the seizure lasting approximately 3 to 5 minutes with the features of generalized tonic clonic convulsion without accompanying a febrile disease. His seizure was not associated with loss of consciousness, postictal confusion or any focal deficits following the seizure episode. There was no history of previous head trauma and his family history was unremarkable. Neurological examination revealed no pathological findings. CT of head was performed at the emergency department to rule out any epileptogenic space occupying lesion but revealed only bilateral small amount of globus pallidus calcifications (Fig. 1).

Fig. 1. Axial nonenhanced CT image of head shows bilateral symmetrical small GPCs (arrows)

He was evaluated by the pediatric neurology department and electroencephalography (EEG) showed normal findings. In addition, magnetic resonance imaging (MRI) was ordered to rule out any subtle structural abnormalities, but revealed normal findings except faint areas of increased signal intensity due to T1 shortening effect of calcifications (Fig. 2) and foci of signal losses on gradient echo (GRE) images (Fig. 3) at bilateral globus pallidus.

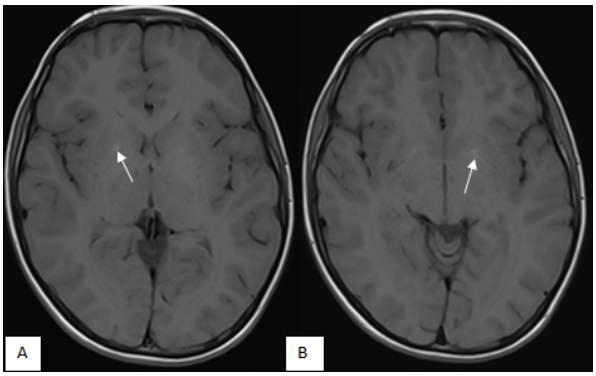

Fig. 2. Axial T1w MRI of brain show faint T1w shortening effect of calcifications on the right (A, arrow) and left (B, arrow) globus pallidum

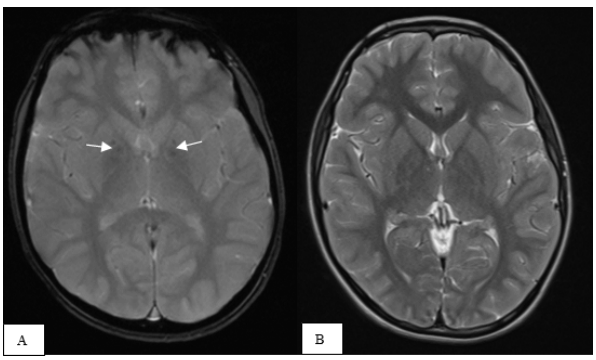

Fig. 3. Axial gradient echo (GRE) image and axial T2w image of the brain. Bilateral globus pallidus calcifications are seen as foci of signal losses on GRE image (A, white arrows). Note that these calcifications are not clearly visible on T2w images.

Initial laboratory results (Table 1) revealed normal hemogram and biochemistry except hypocalcemia (6.58 mg/dL) and hyperphosphatemia (8.23 mg/dL). The patient was referred to the pediatric endocrinology department for further evaluation of the hypocalcemic convulsion, where laboratory investigations revealed idiopathic hypoparathyroidism as the cause of hypocalcemic convulsion with demonstration of low level of serum parathyroid hormone (PTH) (12.2 ng/L). Hypomagnesemia, renal failure and vitamin D deficiency were excluded (Table 1). Renal ultrasonography was performed and ruled out nephrocalcinosis and nephrolithiasis. Ophthalmological examination was normal. DiGeorge syndrome was also excluded by echocardiography and chromosomal analysis.

*Informed consent was taken from the child's parents.

Table 1. The laboratory results

Parameter	Patient's laboratory results	Normal reference
calcium	6.58 mg/dL	8.8-10.8 mg/dL
phosphate	8.23 mg/dL	3-5.4 mg/dL
PTH	12.2 ng/L	15-65 ng/L
magnesium	1.75 mg/dL	1.7-2.1 mg/dL
creatinine	0.45 mg/dL	0.40-0.60 mg/dL
25-hydroxyvitamin D	24 ng/ml	20-40 ng/ml

Discussion

Hypoparathyroidism is an endocrinopathy diagnosed with the laboratory findings of low serum calcium and high serum phosphorus levels associated with decreased or in some instances absent PTH production. Patients present with effects of hypocalcemia including muscle cramps, paraesthesia, seizure, teeth and nail problems. In addition, due to hyperphosphatemia and increased serum phosphorus-calcium products, ectopic tissue calcifications may occur on kidneys, the hearth, vessels, eyes and many other organs and tissues. Intracranial calcifications particularly occurs on basal ganglia and can be associated with epilepsy, cognitive impairment and parkinsonism.[7] The association between BGC and hypoparathyroidism and a correlation between the duration and the severity of the hypocalcemia with BGC were first described by Eaton et al. in 1939.[8] PTH disorders was also reported as the most common definable etiology for white matter and bilateral subcortical nuclei calcifications.[9] Intracranial calcifications of hypoparathyroidism may also involve thalamus, subcortical white matter and corona radiata.[10] In general the most common causes of hypoparathyroidism are iatrogenic and include thyroidectomy & parathyroidectomy or radiotherapy involving the cervical region. In the absence of these situations, in pediatric cases some rare hereditary conditions particularly Di George syndrome and polyglandular autoimmune syndrome type 1 should be considered and excluded.[7] Apart from hypoparathyroidism, vitamin D deficiency/resistance, renal failure and hypomagnesemia may also be the underlying cause and should be excluded in order to accurately manage the hypocalcemic complications. Pseudohypoparathyroidism, the resistance to the action of PTH should be differentiated with its high level of serum PTH. In our patient, low calcium level was associated with the low level of PTH and the high level of phosphorus, consistent with the diagnosis of primary hypoparathyroidism. Serum magnesium, vitamin D and creatinine level were in the normal range.

Conclusion

Since hypoparathyroidism is a treatable cause of hypocalcemic seizure and is associated with multisystemic consequences when untreated, BGCs in pediatric patients should be evaluated with serum electrolyte levels for early diagnosis of hypoparathyroidism and for the prevention of multisystemic complications. Even in small amount of BGC in pediatric patients, as in our case, may be the sign of primary hypoparathyroidism.

References

1. Rastogi R, Beauchamp NJ, Ladenson PW. Calcification of thebasal ganglia in chronic hypoparathyroidism. *J Clin Endocrinol Metab.* 2003;88(4):1476-1477.
2. Perugula ML, Lippmann S. Fahr's Disease or Fahr's Syndrome? *Innovations in Clinical Neuroscience.* 2016;13(7-8):45-46.
3. Morris M, Kwon R, Chen L. Pediatric Idiopathic Basal Ganglia Calcification and Spherocytosis With Chromosome 8p11 Deletion. *J Neuropathol Exp Neurol.* 2020;79(2):238-241.
4. Dähnert W. *Radiology Review Manual.* Wolters Kluwer; 2011.
5. Pistacchi M, Gioulis M, Sanson F, Marsala SM. Fahr's syndrome and clinical correlation: a case series and literature review. *Folia Neuropathologica.* 2016:54(3): 282-294.
6. Amisha F, Munakomi S. *Fahr Syndrome.* Treasure Island (FL): StatPearls Publishing; 2020.
7. Marcucci G, Cianferotti L, Brandi ML. Clinical presentationand management of hypoparathyroidism. *Best Pract Res ClinEndocrinol Metab.* 2018;32:927-939.
8. Eaton LM, Camp JD, Love JG. Symmetric cerebral calcifications, particularly of the basal ganglia demonstrate roentgenographically; calcification of the cerebral blood vessels. *Arch Neurol Psychiatry.* 1939;41:921-942.
9. Manyam BV, Walters AS, Narla KR. Bilateral striopallidodentate calcinosis: clinical characteristics of patients seen in a registry. *Mov Disord.* 2001;16:258-264.
10. Saleem S, Aslam H, Anwar M, et al. Fahr's syndrome: literature review of current evidence. *Orphanet J Rare Dis.* 2013;8:156.

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CASUISTIC PAPER

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Neuroendocrine tumor of appendix located Spiegel hernia – case report and review of the literature

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ABSTRACT

Introduction. Appendix, located within the Spiegel hernia is a rare condition. Few cases have been reported to date. Although it is generally asymptomatic, patients can apply with strangulation findings. Along with the physical examination findings, imaging methods play an important role in diagnosis and definitive diagnosis is made intraoperatively. Per-operative surgical method is determined according to the condition of the structures in the hernia sac. If an appendix is detected in the hernia sac, appendectomy is often preferred regardless of symptoms. Postoperative pathology is mostly benign but malign appendix pathologies should be kept in mind.

Aim. Here, we aimed to present our case undergoing emergency surgery due to incarcerated hernia as it is the first case of appendix neuroendocrine tumor in the Spiegel hernia sac according to our literature review.

Description of the case. A 77-year-old female patient who was admitted to the hospital with complaints of nausea and vomiting was evaluated as an emergency. In the clinical evaluation of the patient, we detected ileus due to hernia. We operated on the patient and found the appendix and cecum in the spiegel hernia. We did appendectomy and hernia repair. Histopathological examination of the appendix revealed a well-differentiated neuroendocrine tumor.

Conclusion. Detection of the appendix in a Spiegel hernia is a rare condition. This is the first case of appendiceal malignancy in a Spiegelian hernia.

Keywords. appendix, carcinoid tumor, hernia, neuroendocrine tumor, Spiegel

Introduction

Spiegel Hernias (SH) are occur on the lateral border of the rectus abdominus muscle and weak fascia on the linea semilinaris. Originally introduced by Josef Klinkosch in 1764, this hernia consists of Adrian van den Spieghel whose name defines linea semilunari. It occurs congenitally or acquired and accounts for 1-2% of all hernias.[1,2]

In the literature, we detected only 22 cases of appendix located the hernia sac of patients operated for SH. As a method, the English language literature based on the MEDLINE (PubMed) database was reviewed. The

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keywords spiegel, hernia and appendicitis were used. None of these cases reported malignancy as a result of histopathological evaluation.

Aim

In this article, we presented the patient, who was operated for SH because of appendix within the hernia sac, in the light of the literature. This case, as far as we know, is the first case in the literature in which a NET was detected in the appendix located in SH.

Description of the case

The patient is an obese women 77 years old. She was admitted to the emergency room with a complaint of abdominal pain, nausea and vomiting. In her past medical history, she had abdominal pain at intervals for the last 1 year. She had a previous history of cholecystectomy and hysterectomy operation. She had no chronic diseases other than hypertension. Body mass index (BMI) was calculated as 36,1. On physical examination, there was minimal distention in the abdomen. There was sensitivity and defense, especially in the lower right quadrant. No mass was detected during palpation. She had gas and outflow. Intestinal sounds were detected normoactive. Gastric fluid came from the nasogastric tube which was about 100 cc. In laboratory tests, WBC: 16.100x10^{3}U/L, CRP: 0.4 were detected. Computed tomography (CT) examination showed that the cecum and appendix were herniated from the 3 cm defect in the lower right side of the rectus muscle in the lower right quadraut (Fig. 1A, B).

Proximal loops were dilated due to compression of the cecum in the hernia sac. Upon this, the patient was operated with a pre-diagnosis of strangulated hernia. The hernia sac was reached through an oblique incision made from the right iliac region. The appendix was found to be adherent in the pouch (Fig. 1C). Appendectomy was performed and the hernia sac was excised. Hernia repair was performed with prolene mesh placed in the preperitoneal area. The patient was discharged from the hospital on the second postoperative day without any problem.

Histologically the tumor was located at the tip of the appendix invading the muscularis propria with a diameter of 5 mm (Fig. 2A, B).

It was composed of solid islands and small groups of round monotonous cells. As there were no mitosis and Ki-67 proliferation index was lower than %1 the tumor was evaluated as well differentiated grade 1 neuroendocrine tumor of the appendix. The tumour cells were diffusely positive for chromogranin and synaptophysin immunohistochemically (Fig. 2C). Mesoappendix and surgical margin were free of tumour.

No recurrence or metastasis was detected during the 2-year follow-up of the patient.

Discussion

SH is a lateral ventral hernia or also called semilunar line hernia . SH arises from the weak area in the Spielian fascia between the rectus muscle and the internal and transverse oblique muscles. Increased intra-abdominal pressure, abdominal wall trauma, and weakening of the fascial layers with age are the reasons included in the etiology.[1,3] Obesity, chronic obstructive pulmonary disease, diabetes, coronary artery disease and peripheral vascular diseases are the most common comorbidities. In addition, 50% of these patients have a history of abdominal surgery.[4] Omentum (39.1%), small intestine (33.7%) and colon (13.5%) are frequently located in the hernial sac. Rarely, the stomach, gallbladder, ovary and appendix can be located in the hernia.[4] Clinical findings may differ according to the structures in the hernia. Patients can usually apply only with pain without palpable swelling, and this makes diagnosis difficult.[5] Our patient also presented with a non-specific abdominal pain without swelling as stated in the literatüre. Ultrasound (US) and CT help in diagnosis. In the series of 81 cases by Larson et al., 74%

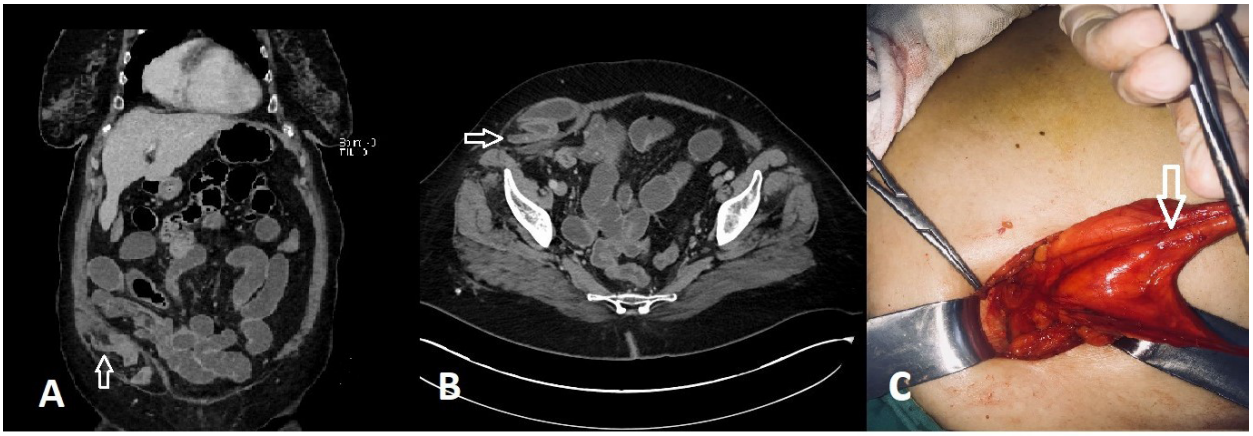

Fig. 1. The coronal CT (A) and axial CT (B). The arrows indicate the insert within the SH sac. Peroperative appendix and mesoapandix (C). The marked arrow shows the appendix inside the opened hernia sac

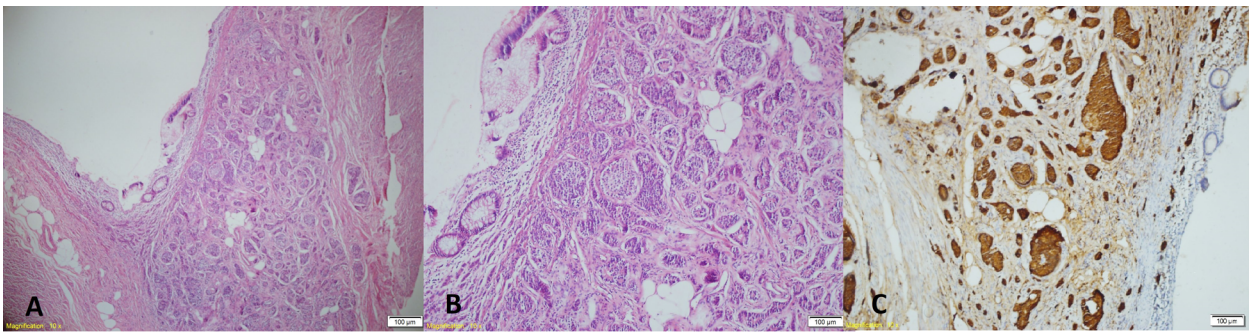

Fig. 2. Histopathologically solid islands and small round uniform cell groups (A: H&E×4, B: H&E×10), Commonly positive tumor cells for chromogranin and synaptophysin (C: Chromogranin×10)

of the patients were diagnosed only by physical examination, while the rest was diagnosed by imaging methods. In this study, incarceration rates in SH were between 17-24%, and 10% of patients were taken to emergency operation.[3] In our patient, diagnosis was made with CT an emergency operation was planned.

Amyand's hernia is the placement of the appendix in the inguinal hernia and named by Claudius Amyand in 1735. De Garengeot hernia is the placement of the appendix in the femoral hernia and it was defined by Rene Jacques Croissant de Garengeot in 1731. The incidence of both is less than 1% of inguinal and femoral hernias.[1,6] The presence of appendix in SH is much more rare also and has not yet been specifically named in the literature. There are only 22 cases.

The purpose of the surgical operation is appendectomy and hernia repair. Appendectomy according to the findings of intraabdominal sepsis and ischemia; it can be done by transabdominal or herniotomy. Open and laparoscopic techniques using primary suture or mesh are applied for SH repair. A number of authors state that the open approach is appropriate in the case of incarceration.[2] In the absence of appendix inflammation in SH, the use of mesh in prophylactic appendectomy and hernia repair remains controversial. There are not enough studies on this subject yet. In the prospective multicenter study of Malazgirt et al., it has been revealed that in open or laparoscopic methods, preperitoneal repair reduces the length of hospital stay and provides better patient compliance.[7] Since our patient had strangulation findings, laparoscopic method was not preferred.

NET which is the most common malignancy of the appendix, is mostly asymptomatic and is detected incidentally after appendectomy. The rate of NET detection in patients undergoing appendectomy is 0.3-0.9%.[8] The vast majority are less than 1 cm and appendectomy is sufficient. In the pathological examination of our case, the treatment was completed in accordance with the 2017 NCCN recommendations, due to the detection of a well-differentiated NET with a diameter of 0.5 cm at the tip of the appendix.[9]

Conclusion

Detection of appendix within the SH sac has not been named before. Our patient is the first case with appendix malignancy in SH. The possibility of malignancy should be kept in mind in these patients. Appendectomy should be performed routinely in case of appendix detected in SH pouch. Primarily laparoscopy should be preferred according to strangulation status. It would be appropriate to remove the appendix using an endobag.

References

1. Klınkosch JT. *Programma quo divisionem herniarum, novamque herniae ventralis speciem proponit*. Clauser, Nobel Press:1764.
2. De la Hermosa AR, Prats IA, Liendo PM, Noboa FN, Calero AM. Spigelian hernia. Personal experience and review of the literature. *Revista Espanola de Enfermedades Digestivas.* 2010;102(10):583-586.
3. Larson DW, Farley DR. Spigelian Hernias: Repair and Outcome for 81 Patients. *World J Surg.* 2002;26(10):1277-1281.
4. Kaliszewski K, Kozakiewicz M, Lubieniecka B, Bronowicki J, Szelachowski P. Spigelian Hernia Containing an Incarcerated Appendix-A Case Report and Literature Review. *J Clin Case Rep.* 2017;7:12.
5. Rankin A, Kostusiak M, Sokker A. Spigelian hernia: case series and review of the literature. *Visceral medicine.* 2019;35(2):133-136.
6. Cheung YF, Ng DCK, Li RSK, Leong HT. Appendicitis in abdominal wall hernia: Case series and literature review. *Surgical Practice.* 2015;19(2):86–89.
7. Malazgirt Z, Topgul K, Sokmen S, et al. Spigelian hernias: a prospective analysis of baseline parameters and surgical outcome of 34 consecutive patients. *Hernia.* 2006;10(4),326–330.
8. Tchana-Sato V, Detry O, Polus M, et al. Carcinoid tumor of the appendix: a consecutive series from 1237 appendectomies. *World J Gastroenterol.* 2006;12(41):6699-6701.
9. NCCN clinical practice guidelines in oncology: neuroendocrine tumors. National Comprehensive Cancer Network web site. https://www.nccn.org/professionals/physician_gls/p df/neuroendocrine.pdf. Accessed June 27, 2017.

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CASUISTIC PAPER

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Posttraumatic arachnoid cyst rupture and delayed acute subdural hygroma

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ABSTRACT

Introduction. Subdural hygromas (SDGs) are the accumulation of fluid in the subdural space. Arachnoid cysts (ACs) on the other hand are common cerebrospinal fluid (CSF) containing lesions of the central nervous system, located within the subarachnoid space. They are generally found incidentally on neuroimaging studies and remain asymptomatic throughout the life. Rupture into the subdural space resulting in subdural hygroma (SDG) is relatively rare.

Aim. We aimed to show the importance of the radiological follow up in head trauma patients having large ACs.

Description of the case. We report a case of a 69-year-old male patient with a known large Galassi type III AC, presented to our hospital with traumatic brain injuries and re-presented with acute posttraumatic SDG in association with AC rupture.

Conclusion. This case emphasizes the importance of radiological follow up in head trauma patients having large ACs to reveal and appropriately manage traumatic subdural collections.

Keywords. acute subdural hygroma, arachnoid cyst rupture, delayed subdural collection

Introduction

Subdural hygromas (SDGs) are the accumulation of fluid in the subdural space. They may be encountered in all age-groups depending on the etiology, but are overall most common in the elderly.[1] Arachnoid cysts (ACs) on the other hand are common cerebrospinal fluid (CSF) containing lesions of the central nervous system, located within the subarachnoid space.[2] They are generally found incidentaly on neuroimaging studies and remain asymptomatic throughout the life. Rupture of an AC into the subdural space resulting in subdural hygroma (SDG) is relatively rare and is resulted from trauma or surgical manipulations. In rare instances, it may also occur spontaneously. On radiological imaging, ACs are well circumscribed extra-axial cysts following CSF pattern on computed tomography (CT) and magnetic resonance imaging (MRI) without restricted diffusion. The ruptured ACs are associated with the same CSF density/intensity fluid extending into the subdural space resulting in a SDG.

Aim

We aimed to show the importance of the radiological follow up in head trauma patients having large ACs.

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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Description of the case

We report a case of a 69-year-old male patient presented to our hospital with scalp hemorrhages, headache and confusion following falling from the ladder on the roof of his house. On examination, there were left periorbital ecchymosis and multiple scalp lacerations. Neurological examination showed a Glasgow coma score (GCS) of 13 (eyes:3, verbal:4, motor:6) without focal neurological deficits. Initial CT of head showed left frontotemporal subgaleal hematoma (Fig. 1A) and subcortical hemorrhagic contusions with focal subarachnoid hemorrhages (SAHs) (Fig. 1B, 1C) on the right frontotemporal lobes, representing countercoup injury. There were also mega cysterna magna (Fig. 1A, 1B), cavum septum pellucidum (Fig. 1C) and a large middle cranial fossa AC (Galassi type III) compressing the temporal lobe on the left side (Fig. 1A, 1C). Scalp lacerations were sutured and he was hospitalized for close follow up. On day 3, the GCS was 15 with CT scan showing no extra axial fluid collection (Fig. 1D). Since the patient was asymptomatic from traumatic brain injuries (TBIs), he was discharged from the hospital with recommendations.

After 1 week, he was re-presented with headache and altered consciousness with CT scan demonstrating bilateral hypodense (similar to CSF) subdural fluid collections (SDFs) on the cerebral convexities, more prominent on the left side where it was associated with compression of the adjacent brain and a slight ventricular compression without midline shift (Fig 2A, 2B).

On 3-tesla MRI of the brain the SDF collections were found to have the same intensity as CSF with homogeneous appearance (Fig 3A, 3B).

The diagnosis of acute posttraumatic subdural hygroma (SDG) was made. Since the patient was symptomatic from the SDG, bilateral burr hole evacuation under general anesthesia (on the left 2 burr hole; frontal and temporoparietal, on the right 1 burr hole; frontal) was performed. The drained fluid was slightly xantochromic

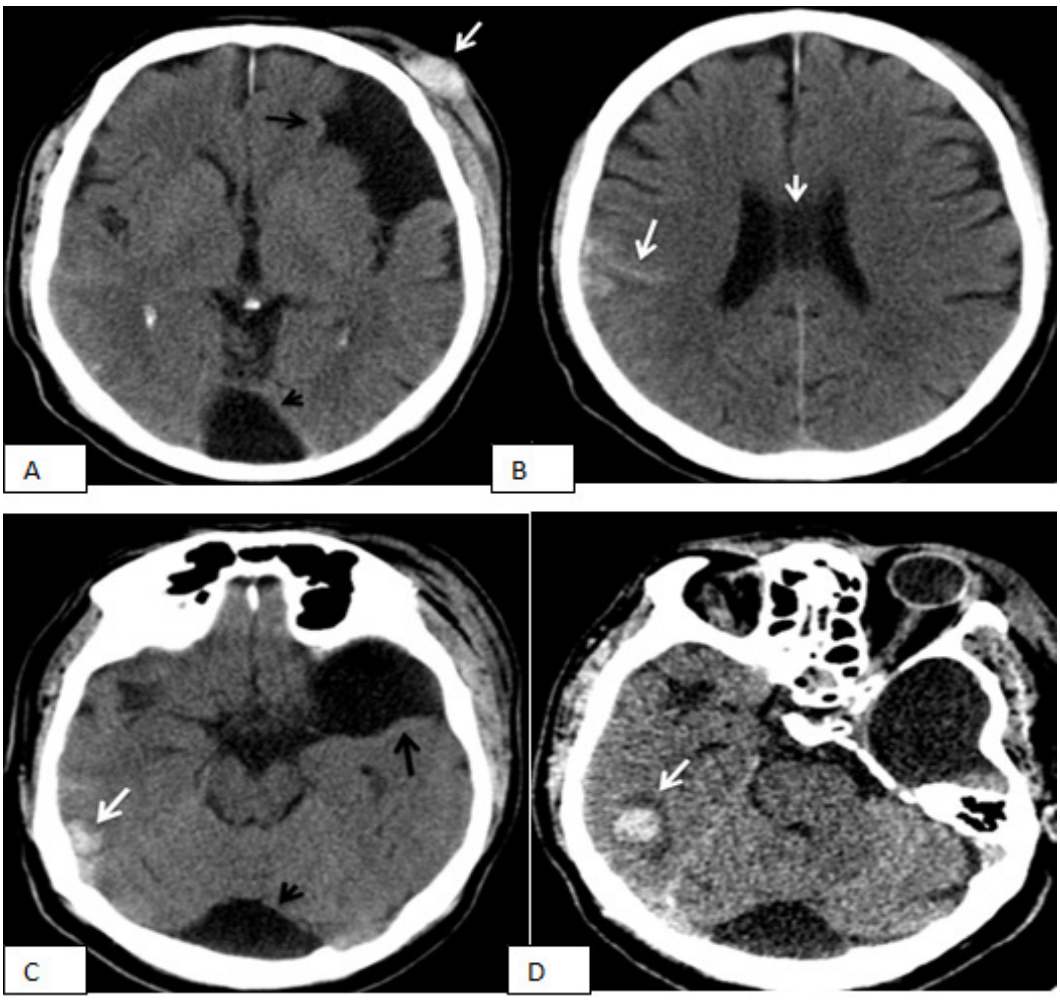

Fig. 1. Initial head CT images show the left frontotemporal subgaleal hematoma (A, white arrow), hemorrhagic contusions and focal subarachnoid hemorrhages at the right frontal lobe (B, white arrow) and the right temporal lobe (C, white arrow). Note the presence of a large middle cranial fossa AC compressing the temporal lobe on the left side (A, C, black arrow), mega cysterna magna (A, B, short black arrow) and cavum septum pellucidum (c, short white arrow). Follow-up CT scan after 3 days from the trauma shows the hemorrhagic contusion which is more prominent due to the surrounding edema (D, white arrow)

supporting its origin of CSF. Postoperatively, he recovered uneventfully and was discharged on the 3th postoperative day by removing the drains with control CT examination. 2 weeks later, in the radiological examination of the patient who came for control evaluation, it was found that the right collection disappeared completely, but the collection on the left increased (Fig 4). There was no neurological deficit but, he had a headache. Considering that this was due to the rupture of the left middle cranial fossa AC, a shunt catheter extending from the existing burr hole into the arachnoid cyst from the subdural area was placed and this was connected to the abdominal cavity. In the subsequent follow-ups, the clinical findings and complaints of the patient recovered completely, and regression was found in the radiological control examinations.

Discussion

SDG is one of the main associated complications of TBIs, most frequently hemorrhagic cerebral contusions or subarachnoid hemorrhages (SAHs).[3-5] However, in these cases, SDG is expected to be more prominent at the same side with these TBIs. Traumatic rupture of existing middle cranial fossa ACs into the subdural space may also be the underlying cause of the acute traumatic SDG. Since the SDF collection was more prominent near the AC in our patient, we thought rupture of the AC as the main cause of the SDG. On head CT scans, SDG appears as a crescentric CSF density accumulation in the subdural space that does not extend into the sulci, usually located along the supratentorial cerebral convexity in frontal or frontoparietal regions.[6] If the

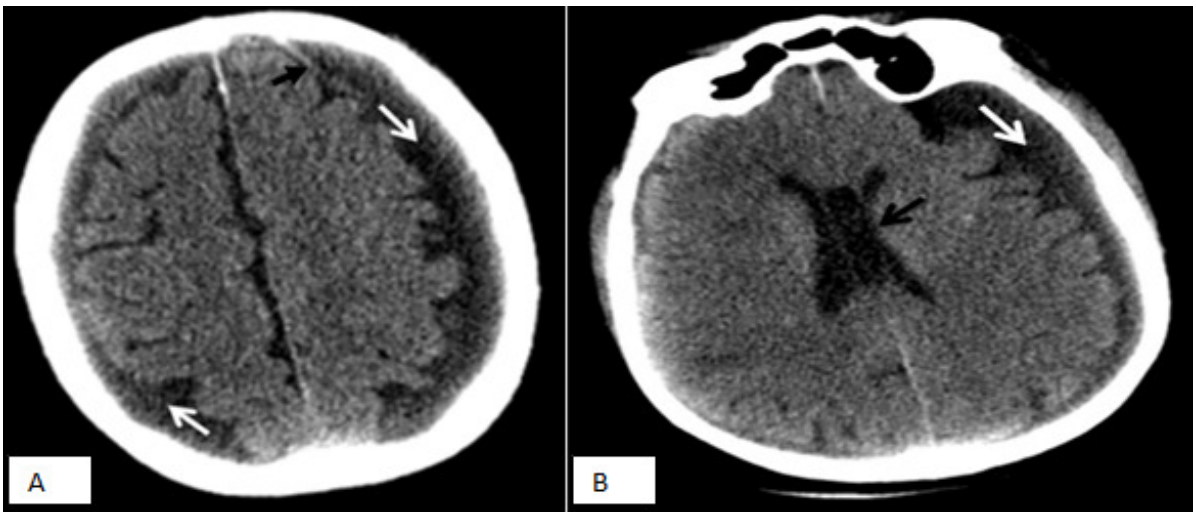

Fig. 2. CT images 1 week after the trauma show bilateral newly developed hypodense SDF collection (A, B, white arrows) on the cerebral convexities which is more prominent on the left side and associated with compression of the adjacent brain (A) and a slight ventricular compression (B, black arrow) without midline shift. Note the presence of vessel crossing the SDF collection (A, black arrow)

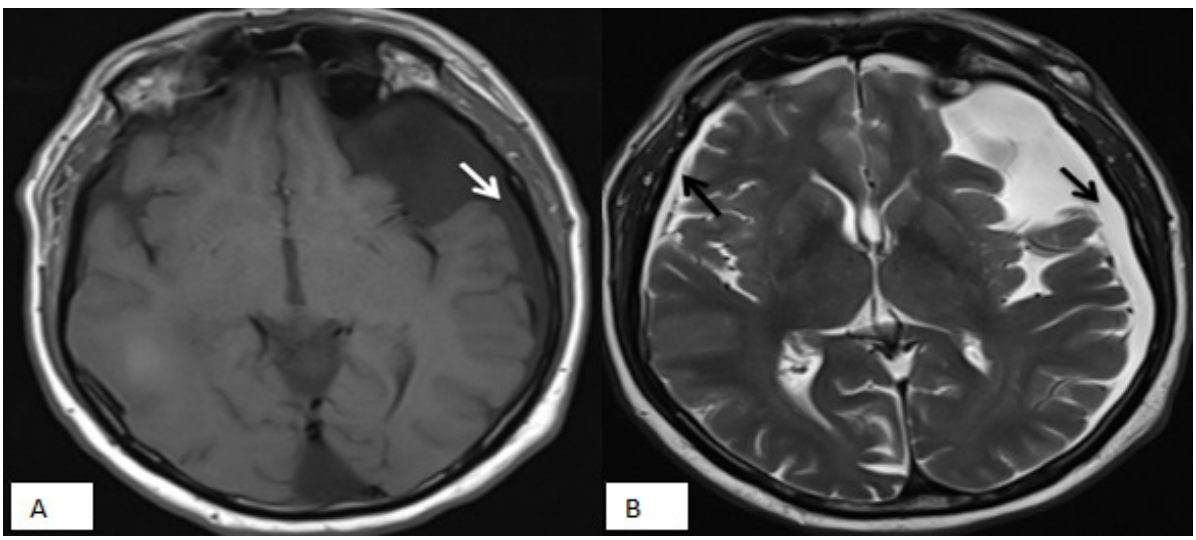

Fig. 3. MRI shows the SDF collections having homogeneously same signal intensities with the CSF on T1w (A, white arrow) and T2w (B, black arrow) images

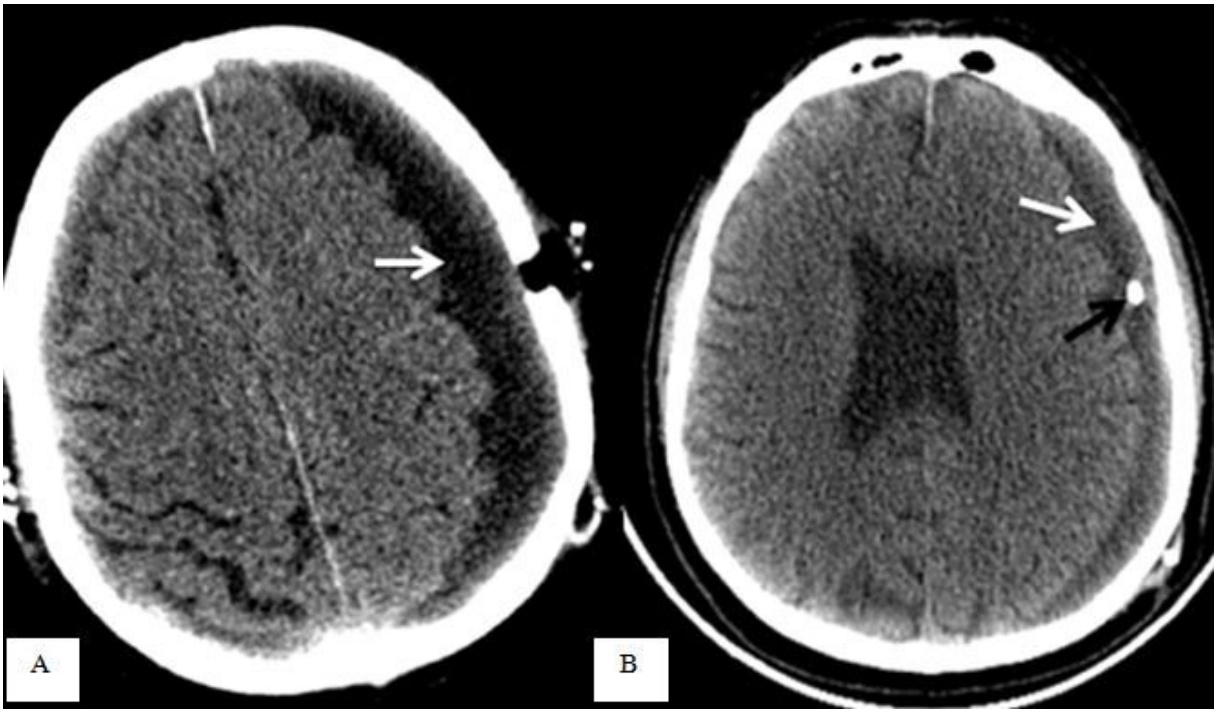

Fig. 4. Follow-up CT scan after burr hole evacuation showing obvious decrease in the right side collection but increase in the left side collection (a, white arrow). Follow-up CT scan after shunt placement (b, black arrow) showing decrease in the amount of left sided collection (b, white arrow).

symptomatic posttraumatic SDF collection is diagnosed as SDG with its characteristic radiological imaging findings and if the patient is symptomatic from the SDG, subduroperitoneal shunt is the first and effective method of management. Due to their identical appearance on CT scans, the main differential diagnosis is chronic subdural hematoma (CSDH) which appear as also near CSF density collection due to the liquefaction of the blood clot.[1-7] However, by definition CSDH is at least 3 weeks of age in contrast to the age of the SDF collection in our patient which was less than or equal to 1 week old. In addition, presence of vessels crossing the SDF collection distinguishes SDG from CSDH and also from cerebral atrophy.[8]

Conclusion

Traumatic rupture of existing middle cranial fossa ACs into the subdural space may be the underlying cause of the acute traumatic SDG**.** Therefore, radiological follow up in head trauma patients having large ACs is important to reveal and appropriately manage traumatic subdural collections.

References

1. Lee KS. History of Chronic Subdural Hematoma. *Korean J Neurotrauma.* 2015;11(2):27-34.
2. Osborn AG, Preece MT. Intracranial cysts: radiologic-pathologic correlation and imaging approach. *Radiology.* 2006;239(3):650-664.
3. Yousefzadeh-Chabok S, Babaei Jandaghi A, Kazemnejad-Leili E, Kouchakinejad-Eramsadati L, Hosseinpour M. Subdural Hygroma in Head Trauma Patients Admitted to a Hospital in Northern Iran. *IrJNS.* 2017;2(4):15-17.
4. Yang XF, Wen L, Shen F, et al. Surgical complications secondary to decompressive craniectomy in patients with a head injury: A series of 108 consecutive cases. *Acta Neurochir (Wien).* 2008;150:1241-1247.
5. Hamamoto Filho PT, Ribeiro Pereira TM, Zanini MA. Epidemiological Features of Patients with Subdural Hygroma in a Prospective Case Series. *Arquivos Brasileiros de Neurocirurgia: Brazilian Neurosurgery.* 2017;36(4):203-206.
6. Fujioka S, Matsukado Y, Kaku M, Sakurama N, Nonaka N, Miura G. CT analysis of 100 cases with chronic subdural hematoma with respect to clinical manifestation and the enlarging process of the hematoma (author's transl). *Neurol Med Chir (Tokyo).* 1981;21:1153-1160.
7. Lee KS. Natural history of chronic subdural haematoma. *Brain injury.* 2004;18(4):351-358.
8. Mccluney KW, Yeakley JW, Fenstermacher MJ, et al. Subdural hygroma versus atrophy on MR brain scans: "the cortical vein sign". *AJNR Am J Neuroradiol.* 1992;13 (5):1335-1339.

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CASUISTIC PAPER

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Anomalous origin of the left vertebral artery from the arch of the aorta

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ABSTRACT

Introduction. Although there are lots of varieties of aorta arch, they seem to appear relatively rarely. Anomalies of aortic arch departures are mainly concerned with its location, course, the place of departure and number of its main branches. However, they warrant attention due to their importance in operative, diagnostic, and endovascular procedures.

Aim. We want to present here a case of a female cadaver with rare aortic arch origin of the left vertebral artery. The aim of this article is to complete the frame of anomalies in aortic arch and to explore rare variability shown in this specific case.

Description of the case. The present report describes an anomalous case of the left vertebral artery arising from the aortic arch between the left common carotid artery and the left subclavian artery in a female cadaver during dissection in an anatomical laboratory. Aortic origin of the vertebral artery is a rare anatomic variant.

Conclusion. Thorough knowledge of anomalous origin is important for patients who undergo operation of an aortic arch or inferior part of the neck. Normally, the vertebral artery arises from the first part of the subclavian artery on both sides.

Keywords. anatomy, dissection, vertebral artery

Introduction

Although there are lots of varieties of aorta arch, they seem to appear relatively rarely. Anomalies of aortic arch departures are mainly concerned with its location, course, the place of departure and number of its main branches.[1,2] Three trunks leave the aortic arch: brachiocephalic trunk, left common carotid artery, left subclavian artery (counted from the right side).[3] Vertebral artery is the first branch which comes from the ascending part of the subclavian artery.[4,5] It goes upwards, covered by longus col-

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li muscle, then comes into foramen of transverse process of C-6 vertebra and goes through foramina of transverse processes of C-5, C-4, C-3 and C- 2, it makes an arch to the foramen of atlas (C-1), creates a sulcus there, in the end enters foramen magnum of the occipital bone. On the clivus inside the cranium, the right and the left vertebral artery join into basilar artery, which divides to upper cerebellar arteries and then posterior cerebral arteries, which are part of the cerebral arterial circle.[4,6]

Aim

We want to present here a case of a female cadaver with rare aortic arch origin of the left vertebral artery. The aim of this article is to complete the frame of anomalies in aortic arch and to explore rare variability shown in this specific case.

Fig. 1. Left vertebral artery, the part which leaves aorta arch between left common carotid artery and left subclavian artery and goes to the transverse process of vertebra C-5, length: 9 cm (vertebral artery marked with pins)

Description of the case

The dissection revealed rare variability of vertebral artery: the vertebral artery departure directly from the aortic arch between left common carotid artery and left subclavian artery. Furthermore, we found a variability of the course in transverse vertebrae processes: it enters the transverse process of C-5 (not C-6 as normal). The variability of the departure of vertebral artery from the aortic arch is noticed more often on the left side. Common is also a situation when the artery enters into foramen of C-4 or C-5 transverse process, where we can find characteristic cusp on that vertebrae.

In our case we divided the vertebral artery into parts:

1st part which goes from the aorta arch to foramen of transverse process of C-5 (length: 9cm) – Fig 1,

2nd part goes through foramina of transverse processes of C-5, C-4 and C-2 (length: 6 cm) – Fig 2,

3rd – the arch that enters foramen of transverse process of first vertebrae (length: 2 cm),

4th part that enters to the foramen magnum of occipital bone.

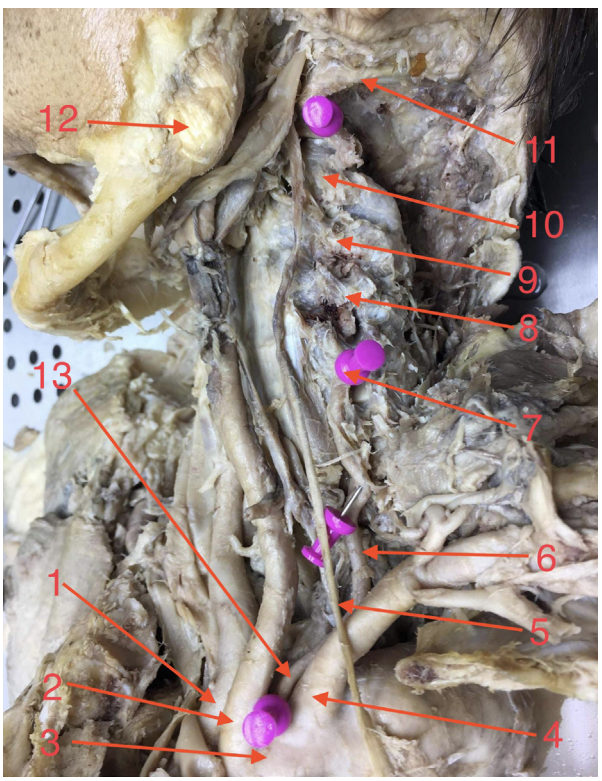

Fig. 2. Left vertebral artery, the part which goes through foramina of transverse processes of vertebrae C-5, C-4 and C-2.
1 – Brachiocephalic trunk, 2 – left common carotid artery, 3 – aortic arch, 4 – left subclavian artery, 5 – left vagus nerve, 6 – left vertebral artery, 7 – left transverse process of C5, 8 - left transverse process of C4, 9 - left transverse process of C3, 10 - left transverse process of C2, 11 – basement of occipital bone, 12 – left angle of mandible, 13 - left vertebral artery (vertebral artery marked with pins)

Discussion

The early detection of anomalies related with artery ramification from aortic arch play significant role in prevention of complications occurred after operation of an aortic arch or inferior part of the neck.[7,8,9,10] They are usually discovered pre-operatively in CT angiography. Clinicians should be aware of the fact that there are more cases

with left aberrant vertebral arteries than right ones.[11,12] Patients with abnormal departure of vertebral artery are usually asymptomatic.[12,13] Furthermore, there is a significant correlation in presence of aneurysm, vascular malformations, pain in occipital regions or in presence of Moyamoya disease connected with abnormalities of vertebral artery ramification.[11,12,14,15,16] The cause of this correlation is not fully understood, it is suspected to have two starting points. The first one applies to congenital abnormalities of arterial structure, the second is connected with modification of intracerebral circulation.[11,17]

In the case of vertebral artery ramification directly from an aortic arch significantly more often this artery is ascending into transverse foramen of cervical vertebrae C5-C6.[5,18] The upper point of entry of vertebral artery into transverse foramen, what may disturb circulation dynamics in the intracranial part, what lead to characteristic symptoms among people with similar anomalies.[11,19,20]

The case presented shows the course of the left vertebral artery with its anomalies placed in the direct departure from the aortic arch. The first variability we noticed during dissection was direct departure of left vertebral artery from aortic arch (the proper place of left vertebral artery departure is left subclavian artery). The second was the left vertebral artery's course variability – it entered into the C-5 transverse process foramen (normally it should first enter the foramen of transverse process of C-6). The final part of the vertebral artery's course in our case seemed not to have any variability and therefore it can be said that blood flow to the brain was not disturbed.

Conclusion

To prevent complications during surgery of the aortic arch or lower neck, recognition and reporting of these variations is important during interpretation of CT angiography. It is also important to recognize anomalous origin of the vertebral arteries before transfemoral catheterization to the vertebral arteries to reduce catheterization failure.

References

1. Kau T, Sinzig M, Gasser J, et al. Aortic development and anomalies. *Semin Intervent Radiol*. 2007;24(2):141-152.
2. Blackwelder H, Madueme P, Dadlani G, Ivsic, T. Multi--modality assessment of the aortic arch branching and vascular rings. *Porg Pediatr Cardiol*. 2020;58:1-12.
3. Shahoud JS, Miao JH, Bolla SR. Anatomy, Thorax, Heart Aorta. 2020 Jan [Updated 2020 Aug 15]. https://www.statpearls.com/
4. Paluszek P, Pieniążek P, Musiałek P. Angioplastyka objawowych zwężeń tętnic kręgowych z zastosowaniem stentów konwencjonalnych i pokrytych lekiem antymitotycznym. *Post Kardiol Interw* 2009;1(15):1-6.
5. Uchino A, Saito N, Takahashi M, et al. Variations in the origin of the vertebral artery and its level of entry into the transverse foramen diagnosed by CT angiography. *Neuroradiology*. 2013;55(5):585-594.
6. Prince EA, Ahn SH. Basic vascular neuroanatomy of the brain and spine: what the general interventional radiologist needs to know. *Semin Intervent Radiol*. 2013;30(3):234-239.
7. Priya S, Thomas R, Nagpal P, Sharma A, Steigner M. Congenital anomalies of the aortic arch. *Cardiovasc Diagn Ther*. 2018;8(1):26-44.
8. Uchino A, Saito N, Watadani T, et al. Vertebral artery variations at the C1-2 level diagnosed by magnetic resonance angiography. *Neuroradiology*. 2012;54(1):19-23.
9. Vučurević G, Marinković S, Puškaš L, et al. Anatomy and radiology of the variations of aortic arch branches in 1,266 patients. *Folia Morphol (Warsz)*. 2013;72(2):113-122.
10. Wakao N, Takeuchi M, Kamiya M, et al. Variance of cervical vertebral artery measured by CT angiography and its influence on C7 pedicle anatomy. *Spine (Phila Pa 1976)*. 2014;39(3):228-332.
11. Komiyama M, Morikawa T, Nakajima H, Nishikawa M, Yasui T. High incidence of arterial dissection associated with left vertebral artery of aortic origin. *Neurol Med Chir*. 2001;41(1):8-12.
12. Yuan SM. Aberrant Origin of Vertebral Artery and its Clinical Implications. *Braz J Cardiovasc Surg*. 2016;31(1):52-59.
13. Bravo C, Gámez F, Pérez R, Álvarez T, De León-Luis J. Fetal Aortic Arch Anomalies: Key Sonographic Views for Their Differential Diagnosis and Clinical Implications Using the Cardiovascular System Sonographic Evaluation Protocol. *J Ultrasound Med*. 2016;35(2):237-251.
14. Beyaz P, Khan N, Baltsavias G. Multiple anomalies in the origin and course of vertebral arteries and aberrant right subclavian artery in a child with moyamoya syndrome. *BMJ Case Rep*. 2018;2018:bcr-2017-013464.
15. Ali A, Abbas M, Saeed U. A rare cause of stroke in young: moyamoya disease. *Postgraduate Medical Journal*. 2020;96:119.
16. Zhang H, Zheng L, Feng L. Epidemiology, diagnosis and treatment of moyamoya disease. *Exp Ther Med*. 2019;17(3):1977-1984.
17. Meila D, Tysiac M, Petersen M, et al. Origin and course of the extracranial vertebral artery: CTA findings and embryologic considerations. *Clin Neuroradiol*. 2012;22(4):327-333.
18. Arslan M, Acar HI, Comert A, Tubbs RS. The Cervical Arteries: An Anatomical Study with Application to Avoid the Nerve Root and Spinal Cord Blood Supply. *Turk Neurosurg*. 2018;28(2):234-340.
19. Chandra A, Li WA, Stone CR, Geng X, Ding Y. The cerebral circulation and cerebrovascular disease I: Anatomy. *Brain Circ*. 2017;3(2):45-56.
20. Cagnie B, Barbaix E, Vinck E, D'Herde K, Cambier D, Dirk. Extrinsic risk factors for compromised blood flow in the vertebral artery: Anatomical observations of the transverse foramina from C3 to C7. *Surg Radiol Anat*. 2005;27:312-316.

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LETTER TO THE EDITOR

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Ivermectin for COVID-19 prophylaxis

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Dear Editor,

With the advent of COVID-19 globally, many doctors have begun to review the medications that are already available to treat and/or protect against the infection.[1] One of these drugs is the antiparasitic ivermectin. The first clinical trial was registered by Zagazig University in May, 2020; and hence, it has been adopted in several studies.[2-5]

Ivermectin is one of the medicines previously listed by WHO as one of essential drugs; and approved by US Food and Drug Administration (FDA) as an antiparasitic drug.[6] It is a 50-years-old generic drug. It has been used with a long history of safety for about 4 billion times around the world.[7] It has been widely administered against many parasitic diseases including strongyloidiasis, onchocerciasis and lymphatic filariasis.[8] Ivermectin is an inexpensive, readily available drug that is well-accepted for use by some health authorities around the world on large scales. It can also be easily taken by mouth. Moreover, ivermectin administered even with a high dose up to 2000 μg/kg is well-tolerated. Such dose represents up to 10 times the highest FDA-approved dose of 200 μg/kg.[9,10] Despite the evident safety of ivermectin, it is contraindicated in patients with blood-brain barrier (BBB) leakage such as in cases of meningitis.[11] Regarding its use for pregnant women, infants and children below than 15 kg body weight, there is insufficient evidence for safety.[12,13] Therefore, its use for those categories of people might be avoided.

It has been promoted to be used in large scales in India with great success.[14] Moreover, it has been investigated in countries of Latin America, South Africa and others with encouraging reports. Meanwhile, it has been taken up by self-medicated individuals in many countries including USA. Although, it is not WHO or FDA-approved drug for COVID-19, a recent study stated that there is an increase in use of ivermectin by American citizens during pandemic period that peaked in January 2021 with a relative increase of 989%.[15] This might be an important factor other than the widespread distribution of vaccines in reducing the number of cases of COVID-19. At the same time, use of ivermectin in Peru resulted in an approximately 14-fold decrease in excess deaths versus a 13-fold increase with reversed ivermectin policy.[7]

Ivermectin could be taken at home under medical supervision at appearance of the first signs of

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

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COVID-19, and it can also be given to individuals who are in close contact with confirmed cases. It might significantly reduce COVID severity and progression especially if administered as early as possible. Subsequently, the numbers of cases could be reduced; and therefore, fewer patients will need to be hospitalized. Its use in protection might also be based on the reports stating that it is of great benefit in treating cases of mild and moderate cases. However, no benefit for use has been reported for use in severe cases who are in need for mechanical ventilation.[16]

In vitro studies, ivermectin has been observed to inhibit the replication of SARS-CoV-2 "the virus causing corona". It is considered a host-directed antiviral agent so it can be of a broad-spectrum antiviral including viruses causing Zika, dengue, HIV and yellow fever.[17] It has been stated that ivermectin could prevent protein of virus from entering host cell nucleus.[18] Moreover, its binding with SARS-CoV-2 spike protein is non-epitope specific. Therefore, it might be effective for combating the mutations and different variants of the SARS-CoV-2.[7] Moreover, Zaidi and Dehgani-Mobaraki explained 20 different mechanisms for ivermectin against viruses including direct actions on the virus as well as effect on the host cells to resist viral replication and to target the virus-induced inflammation.[19] They added that it is of worthy attention to repurpose another approved drug such as ivermectin in order to overcome the new mutant strains of coronavirus and the potential re-emergence of novel viruses. In a report issued by England Ministry of Health on 9 July 2021, it has shown that the numbers of deaths due to the delta variant of the virus were higher among vaccinated persons compared to nonvaccinated cases with sample numbers of 118 versus 92, respectively.[20] This means that the current vaccines may not be effective against new strains of virus.

We call on the experts of WHO to reconsider their advice against use of ivermectin for COVID-19; and instead, they might recommend its urgent investigation especially since WHO has been previously declared that it could be used for mass treatment and prophylaxis in other diseases.[6,21] The attribution of WHO advice against ivermectin use in the current pandemic has been based on argument of insufficient evidences. Despite such advice of WHO, many governments have turned to it for combating the pandemic. However, we see no convincing reason to reject use of ivermectin in the current pandemic despite its high efficacy, safety and ease of its trials and confirmation of its positive effects. Meanwhile, testing of its effectiveness is so easy and doesn't require facilities except to accept volunteers to participate in the investigations without reported health risks or even economic burden.

It is emphasized that no drug or vaccine can be said to eliminate or protect from infections without proper clinical trials. In this context, Bryant et al. performed a meta-analysis of 15 clinical trials conducted up to April 25, 2021 for investigation of efficacy of ivermectin.[22] They concluded that ivermectin could be of great impact in combating SARS-CoV-2 pandemic. Moreover, a recent study conducted by French Pasteur Institute confirmed the good effective role of ivermectin against COVID-19 infection.[23] Accordingly, we agree the opinion of other authors that apparent efficacy, safety and low cost of ivermectin could be an important cause for its significant role in control of the current pandemic globally.[22]

Meanwhile, many encouraging news have come from almost all countries and governments adopting ivermectin for prophylaxis with a dramatic drop in the number of cases. These countries include India, Peru, Mexico, South Africa, Brazil and other countries.[24] Moreover, it has been approved for use against COVID-19 in many other countries including Honduras, Slovakia and Czech Republic.[25]

Some authors have endorsed the use of a prophylactic weekly dose of ivermectin to protect health care workers from infection with the coronavirus.[26] Meanwhile, a group of expert physicians named Front Line COVID-19 Critical Care Alliance reviewed the effects of ivermectin on COVID-19 infections; and concluded that it has showed a strong evidence of therapeutic efficacy and is recommended for prophylaxis and therapy of SARS-CoV-2 infection.[27] In a previous report, we proposed use of ivermectin in mass prophylaxis by administering one or more doses to all members of community at the same time.[5] However, if this is difficult to be adopted by health authorities, ivermectin might be administered at regular intervals for health care members, and as a single booster dose if contact with patients is suspected. Urgent prospective studies on large numbers of cases are recommended to determine the exact protocol and doses to be used in the current pandemic.

Conclusions

Use of ivermectin on a wide scale is very safe as previously stated by WHO in control of some parasitic diseases. It has been tested by numerous clinical trials and researches as a therapeutic and prophylaxis for the current pandemic. It can provide a temporary protection but not prolonged immunization. It might protect people from infection at when exposed to cases of COVID-19. In addition, it eliminates the infections in the early stages of the disease. Even in vaccinated individuals who have had COVID-19 infection, they can use it for treatment. To be effective in COVID-19 prophylaxis, it is suggested to be given to all members of community at the same time. Otherwise, it may be taken when contact with COVID-19 patients or disease-carriers. It is also a call to WHO and US FDA to re-consider their advice against use of ivermectin in prophylaxis of the current pandemic.

References

1. Hegazy AA, Hegazy RA. COVID- 19: Virology, pathogenesis and potential therapeutics. *Afro-Egypt J Infect Endem Dis.* 2020;10(2):93-99.
2. Shouman WM. Prophylactic ivermectin in COVID-19 contacts; ClinicalTrials.gov (2020). Available at: https://clinicaltrials.gov/ct2/show/NCT04422561 (Accessed June 10, 2021)
3. Shouman WM, Nafae RM, Hegazy AA, et al. Use of ivermectin as a prophylactic option in asymptomatic family close contacts for patients with COVID-19 (2020). Available at: https://clinicaltrials.gov/ProvidedDocs/61/NCT04422561/Prot_SAP_000.pdf (Accessed June 10, 2021)
4. Shouman WM, Hegazy AA, Nafae RM, et al. Use of Ivermectin as a Potential Chemoprophylaxis for COVID-19 in Egypt: A Randomized Clinical Trial. *J Clin Diagnostic Res.* 2021;15(2):27-32.
5. Hegazy AA, Alghamdi MS, Shouman WM, Hegazy RA. Mass Chemoprophylaxis with Ivermectin against COVID-19 Pandemic: Review and Authors' Perspective. *Acta Scient Med Sci.* 2021;5(6):47-51.
6. Ahmed S, Karim MM, Ross AG, et al. A five-day course of ivermectin for the treatment of COVID-19 may reduce the duration of illness. *Int J Infect Dis.* 2021;103:214-216.
7. Chamie-Quintero J, Hibberd JA, Scheim D. Ivermectin for COVID-19 in Peru: 14-fold reduction in nationwide excess deaths, p=. 002 for effect by state, then 13-fold increase after ivermectin use restricted. 2021;1-13. doi:10.31219/osf.io/9egh4
8. Ramírez C, Herrera-Paz EF, Peralta G, et al. Is ivermectin ready to be part of a public health policy for COVID-19 prophylaxis? *EClinicalMedicine.* 2021;32:100744.
9. Guzzo CA, Furtek CI, Porras AG, et al. Safety, tolerability, and pharmacokinetics of escalating high doses of ivermectin in healthy adult subjects. *J Clin Pharmacol.* 2002;42(10):1122-1133.
10. Krolewiecki A, Lifschitz A, Moragas M, et al. Antiviral effect of high-dose ivermectin in adults with COVID-19: A proof-of-concept randomized trial. *EClinicalMedicine.* 2021;37:100959.
11. Nobmann S, Bauer B, Fricker G. Ivermectin excretion by isolated functionally intact brain endothelial capillaries. *Br J Pharmacol.* 2001;132(3):722-728.
12. Nicolas P, Maia MF, Bassat Q, et al. Safety of oral ivermectin during pregnancy: a systematic review and meta-analysis. *Lancet Glob Health.* 2020;8(1):e92-e100.
13. Levy M, Martin L, Bursztejn AC, et al. Ivermectin safety in infants and children under 15 kg treated for scabies: a multicentric observational study. *Br J Dermatol.* 2020;182(4):1003-1006.
14. Hope J. Ivermectin saves India | Letter to the Editor. 2021; Available at: https://www.thedesertreview.com/opinion/letters_to_editor/ivermectin-saves-india/article_14b1f1d6-cd2f-11eb-8b78-9710d864f627.html (Accessed June 12, 2021)
15. Lind JN, Lovegrove MC, Geller AI, et al. Increase in Outpatient Ivermectin Dispensing in the US During the COVID-19 Pandemic: A Cross-Sectional Analysis. *J Gen Intern Med.* 2021:1–3. doi:10.1007/s11606-021-06948-6
16. Mathias J. Use of ivermectin for prevention, treatment of COVID-19. 2021; Available at: https://www.ormanager.com/briefs/use-of-ivermectin-for-prevention-treatment-of-covid-19/ (Accessed June 15, 2021)
17. Abreu JL. Ivermectin for the prevention of COVID-19. So…WHO is telling the Truth? *Daena: Int J Good Cons.* 2020;A2.15(2)1-30.
18. Caly L, Druce JD, Catton MG, et al. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res.* 2020;178:104787.
19. Zaidi AK, Dehgani-Mobaraki P. The mechanisms of action of Ivermectin against SARS-CoV-2: An evidence-based clinical review article. *J Antibiot (Tokyo).* 2021:1–13. doi:10.1038/s41429-021-00430-5
20. Public Health England. SARS-CoV-2 variants of concern and variants under investigation in England Technical briefing 18; 9 July 2021. Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1000678/Variants_of_Concern_VOC_Technical_Briefing_18.pdf
21. Speare R, Durrheim D. Mass treatment with ivermectin: an underutilized public health strategy. *Bull World Health Organ.* 2004;82(8):562.
22. Bryant A, Lawrie TA, Dowswell T, et al. Ivermectin for Prevention and Treatment of COVID-19 Infection: A Systematic review, meta-analysis, and trial sequential analysis to inform clinical guidelines. *Am J Ther.* 2021. doi:10.1097/MJT.0000000000001402
23. de Melo GD, Lazarini F, Larrous F, et al. Attenuation of clinical and immunological outcomes during SARS-CoV-2 infection by ivermectin. *EMBO Mol Med.* 2021;25:e14122.
24. Fox 40. Front line COVID-19 critical care alliance to Japanese Olympic Committee: The Tokyo Olympic Games Can Go on Safely. 2021; Available at: https://www.wicz.com/story/44037795/front-line-covid-19-critical-care-alliance-to-japanese-olympic-committee-the-tokyo-olympic-games-can-go-on-safely
25. Full fact. Ivermectin for Covid-19 remains controversial. 2021; Available at: https://fullfact.org/health/ivermectin--covid-treatment/
26. Morgenstern J, Redondo JN, Olavarría Á, et al. Retrospective cohort study of Ivermectin as a SARS-CoV-2 pre--exposure prophylaxis method in Healthcare Workers. *MedRxiv.* 2021. doi:10.1101/2021.04.10.21255248
27. Kory P, Meduri GU, Varon J, et al. Review of the emerging evidence demonstrating the efficacy of ivermectin in the prophylaxis and treatment of COVID-19. *Am J Ther.* 2021;28(3):e299.

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