Acta Medica Academica

Journal of Department of Medical Sciences
of Academy of Sciences and Arts of Bosnia and Herzegovina

ISSN 1840-1848 (Print) Volume 51 Number 1 April 2022 ISSN 1840-2879 (Online)

Online First www.ama.ba

Acta Medica Academica

Contents
2022 Vol. 51, No. 1

Clinical Medicine

Acta Medica Academica

www.ama.ba

AIMS AND SCOPE

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SUBSCRIPTION

Acta Medica Academica is published triannually. The annual subscription fee is € 50 outside of Bosnia and Herzegovina.

PUBLISHER CONTACT INFORMATION

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COVER PHOTO PICTURE

Faruk Ibrahimović (1943). Vakuf's Hospital "Hastahana" in Tuzla. The second hospital facility in Bosnia and Herzegovina was built in 1874. Photographed around 1975. Courtesy of the author.

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CIRCULATION

500 copies.

THE JOURNAL IS INDEXED IN

Medline/PubMed; Scopus; Embase; EBSCOhost; Index Copernicus; CAB Abstract/Global Health Databases; IndexScholar.com; DOAJ; CrossRef; InfoBase Index.

Acta Medica Academica (ISSN 1840-1848) is an international peer-reviewed triannual journal which is printed as a continuation of the journal Works of the Academy of Sciences and Arts of Bosnia and Herzegovina, Department of Medical Sciences, founded in 1953. *Acta Medica Academica* Online (ISSN 1840-2879) offers free access to all articles at www.ama.ba.

Original Article
Acta Medica Academica 2022;51(1):1-13
DOI: 10.5644/ama2006-124.364

Non-interventional Pilot Study Evaluating the Efficacy and Safety of Lysozyme-based Therapy in Patients with Non-infectious Sore Throat

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Received: 30 March 2022; **Accepted:** 28 April 2022

Abstract

Objective. This study aimed to evaluate the efficacy and safety of lysozyme-based oral antiseptic in the therapy of non-infectious sore throat in teachers. **Materials and Methods.** A non-interventional, prospective, pilot study was conducted with two examinations. The first was performed as part of a general medical examination. If a non-infectious sore throat was confirmed by clinical checkup and all other inclusion and non-exclusion criteria confirmed, patients were offered to be enrolled in the study. After signing the informed consent form, patients were advised to use lysozyme-based lozenges, six times a day, for a period of five days. A telephone call follow-up examination was performed within 24 hours from the therapy completion. **Results.** This was a pilot study involving 25 adult patients of both genders. Lysozyme-based lozenges showed positive effects in relieving the symptoms of non-infectious sore throat in teachers. At the same time, the lozenges showed excellent tolerability, and no side effects were reported during the study. 92% of patients confirmed they would take the same medicine again due to the same problem. **Conclusion.** The results of this "proof-of-concept" study indicated that lysozyme-based antiseptic could be effective and safe in the treatment of non-infectious sore throat in teachers and should be further evaluated as treatment option in this condition.

Key Words: Lysozyme ▪ Functional Dysphonia ▪ Professional Exposure ▪ Sore Throat.

Introduction

It is estimated that around one-third of workers in the modern world use voice as the principal tool at work. Certain vocally demanding professions such as teachers, doctors, nurses, tour guides, actors and singers are at higher risk of developing throat irritation problems (1, 2). Terms such as throat irritation, functional dysphonia due to professional exposure, and throat discomfort are used as synonyms for non-infectious sore throat (3, 4). The term "non-infectious sore throat" may sometimes be confusing and insufficiently explained, resulting in the inclusion of criteria associated with acute and chronic pharyngitis of microbial etiology in studies dealing with this issue. It has to be emphasized that non-infectious sore throat can be a result of functional dysphonia (3). The problem of functional dysphonia is recognized as an important factor influencing the everyday communication, social, and professional life of affected individuals. It can result in social isolation, increased need for sick leave, and depression (5).

In pre-clinical and clinical models, inflammation and underlying mechanisms of non-infectious sore throat were evaluated. A rat experimental model of pharyngitis induced by pyridine solution

was used to investigate the role of inflammation and immune response in non-infectious sore throat (6). In this model, microscopic changes as well as increased levels of tumor necrosis factor alpha (TNF-α), interleukin - 6 (IL-6) and expression of defensins' genes were observed. Those changes could be mitigated by application of phytochemical preparations with immunomodulatory and anti-inflammatory activities (7, 8). Non-infectious sore throat was investigated in clinical settings as well, when pharynx was exposed to cold dry air and acute response characteristic for non-infectious sore throat was provoked. It caused a feeling of pain and short-term increase of inflammatory parameters (prostaglandin E2, thromboxane B2, and substance P) in pharyngeal lavage fluid (9). In a similar manner, prolonged mouth breathing (10) and obstructive sleep apnea (11) can lead to inflammation.

Although there is no unique doctrine in the treatment, anti-inflammatory medicines and oral antiseptics are usually used along with vocal hygiene (3). Lysozyme is an enzybiotic with immunomodulatory and anti-inflammatory effects (12) that could be utilized in non-infectious sore throat treatment (13). Due to its pharmacological effects and natural origin, lysozyme is an interesting and potentially efficacious treatment of non-infectious sore throat.

The objective of this pilot non-interventional prospective study was to evaluate the results of monitoring the efficacy and safety of lysozyme-based oral antiseptic in the therapy of non-infectious sore throat in teachers.

Materials and Methods

A non-interventional pilot study to evaluate the efficacy and safety of lysozyme-based therapy in patients with non-infectious sore throat was conducted at the Institute of Occupational Medicine, Health Care Centre of Canton Sarajevo, Bosnia and Herzegovina, from July to September 2020. The primary aim was to treat non-infectious sore throat in teachers with lysozyme-based lozenges and evaluate the efficacy of therapy through collection of data using a validated questionnaire (14) for Rating Quality of Life Related to Sore Throat where the answers are rated on the Likert scale from 1 (lowest) to 5 (highest) with exception of the question "Are you able to take care about yourself completely?" where scale from 1 (highest) to 5 (lowest) was used. Secondary aims were to evaluate the safety of lysozyme-based lozenges by monitoring the frequency and types of adverse events during the therapy and to investigate the characteristics of non-infectious sore throat as a result of a secondary reaction to functional dysphonia in teachers.

Patients

The study included patients of both genders, aged 18 years and older, employed as teachers and with symptoms of non-infectious sore throat confirmed on the basis of clinical examination by medical doctor. Non-inclusion criteria were symptoms of infectious sore throat confirmed on the basis of a clinical examination by medical doctor, usage of empirical antibiotic therapy due to a previously diagnosed infectious sore throat, usage of preparations for the treatment of sore throat in the form of sprays or lozenges, malignant disease, hypersensitivity to the active substance and / or excipients of the drug, hypersensitivity to egg whites, pregnancy and lactation. Exclusion criteria were exacerbation of the underlying disease and development of serious adverse events requiring discontinuation of therapy.

Study Drug

After signing the informed consent form, patients were advised to use Lysobact® lozenges (compressed lozenges with smooth undamaged edges and 8 mm diameter containing lysozyme hydrochloride 20 mg and pyridoxine hydrochloride 10 mg as active ingredients and lactose monohydrate, tragacanth, saccharin sodium, magnesium stearate, and vanillin as excipients, producer: Bosnalijek d.d., Bosnia and Herzegovina). Six lozenges were used during the day for a period of five days, according to the valid patient information leaflet (15). It was recommended that lozenges should be allowed to slowly

melt under the tongue with an interval of at least one hour between each intake. A follow-up telephone examination was performed on the sixth day (within 24 hours after the end of therapy).

Data Collection

First examination was performed as part of a general medical examination. Figure 1 shows the monitored parameters. In Supplemental data, the test list used in the study is provided.

Ethical Statement

The study was conducted according to the criteria set by the Declaration of Helsinki of 1975, as revised in 2000, and each patient signed an informed consent before enrolment to the study.

Statistical Analysis

The descriptive statistics was performed and data were presented as absolute values and percentages or as medians and interquartile range (IQR). Wilcoxon signed rank test was used to determine differences in subjective feeling of pain, difficulty swallowing, and the posterior pharyngeal wall swelling at first and follow-up examination. $P<0.05$ was accepted as statistically significant. The statistical analysis was performed in Statistical Package for the Social Sciences (SPSS) IBM Version 26 (SPSS) (UNICOM Systems, Inc.)

Results

25 patients were included, 5 men and 20 women. Patients' baseline characteristics are presented in Table 1.

Table 1. Baseline Characteristics of Patients Involved in the Study. Data Is Presented as Absolute Numbers and Percentages

Characteristics	Total number of patients N=25; (%)
Age (Years)	
50 - 65	6 (24)
30 - 50	18 (72)
18 - 30	0 (0)
Unknown	1 (4)
Gender (Female / Male)	20 (80) / 5 (20)
Work experience (Years)	
<5	0 (0)
5 - 10	6 (24)
10 - 20	10 (40)
20 - 30	7 (28)
>30	2 (8)
Working place	
Primary School	7 (28)
High School	18 (72)

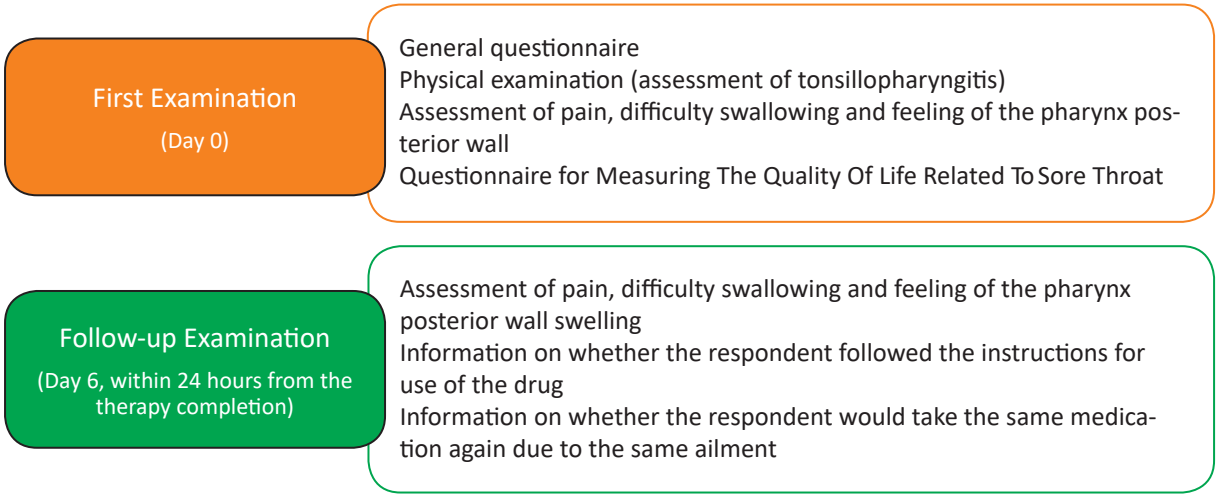


Figure 1. Study design.

First Examination Results

The first examination results were used for evaluation of the characteristics of non-infectious sore throat in teachers. The general questionnaire showed that most patients felt dry throat during the day, used some of the throat preparations, throat problems made them nervous, and throat discomfort decreased when they were silent. On the other side, a small number of patients often visited a doctor due to a sore throat or had a confirmed allergy (Figure 2). Physical examination revealed that none of the subjects had an oropharyngeal enanthema (vesicles, petechiae, exudate). Most patients had normal tonsil size (N=17), two patients had slightly enlarged, two patients had moderately enlarged, one patient had considerably enlarged, while three patients had removed tonsils. The color of the oropharyngeal mucosa was normal in 24 patients, while in one patient it was red.

The questionnaire for measuring the quality of life related to sore throat examined the impact of non-infectious sore throat in the patients through the physical, psychological, social, and environmental domains (Table 2). In the physical domain, it was observed that there was a mild to moderate sore throat, moderate effect on swallowing, little or no effect of sore throat on sleep and breathing, little effect on exhaustion and movement, and that most patients could fully take care of themselves. In the psychological domain, it has been observed that sore throat moderately deconcentrated most of the patients, while it did not make them depressed or affected their daily religious activities. In the social domain, it was noticed that for the majority of patients, sore throat moderately interfered with work, had little interference in relationships with family members and socializing with friends, and almost no interference in monitoring media content or sexual activity. In the domain of the environment, it has been found that sore throat had a small effect on colleagues at work or prevention of others from associating with patients, almost never led to financial losses and endangered personal

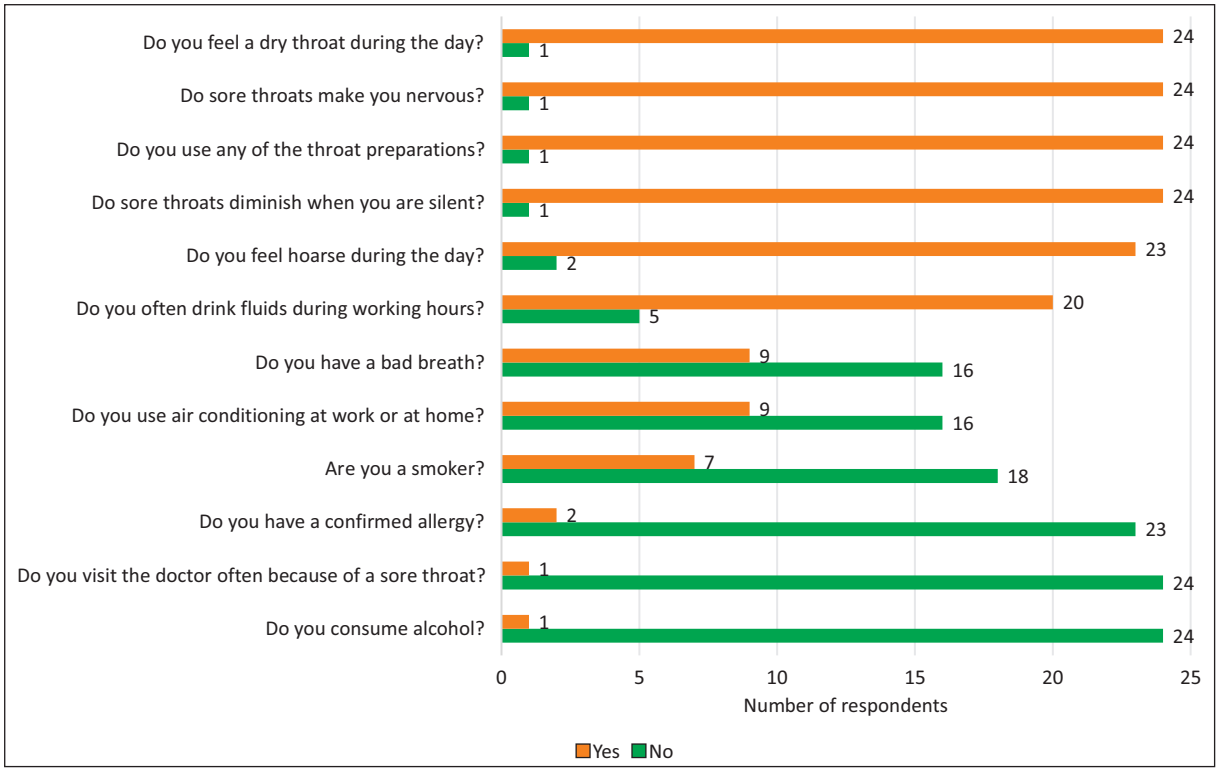


Figure 2. The general questionnaire results.

Table 2. Results of the Questionnaire for Rating Quality of Life Related to Sore Throat. The Answers Are Rated on the Likert Scale from 1 (lowest) to 5 (highest) with Exception of the Question "are You Able to Take Care about Yourself Completely?"Where Scale from 1 (highest) to 5 (lowest) Was Used

Domain	Median	IQR
Physical	2.00	1.00 - 3.00
How much your throat hurts, i.e. "burns" or "scratches"?	3	2 - 3
Does sore throat make swallowing difficult?	3	2 - 3
How much sore throat affects your sleep?	2	1 - 2
Does sore throat make breathing difficult?	2	1 - 2
Do you feel exhausted due to sore throat?	2	2 - 3
Are you able to take care about yourself completely?	1	1 - 1
Do you walk less due to the sore throat?	2	1 - 2
Psychological	1.00	1.00 - 2.50
How much sore throat affects your concentration?	2	2 - 3
Does sore throat make you depressive?	1	1 - 2
Does sore throat interfere with your daily religious activities?	1	1 - 2
Social	2.00	1.00 - 2.00
Does sore throat affect your relations with family members?	2	1 - 2
Does sore throat interfere with your work?	3	2 - 3
Does sore make following media more difficult?	1	1 - 2
Does sore throat interfere with your friendships?	2	2 - 2
Does sore throat interfere with your sexual activities?	1	1 - 2
Environment	2.00	1.00 - 2.75
Does sore throat make your colleagues at work uncomfortable?	2	1 - 2
Do you have financial losses due to the sore throat?	1	1 - 1
Does sore throat impair your personal security?	1	1 - 2
Does your sore throat prevent others to socialize with you?	2	1 - 2
Does sore throat impair your ability to withstand pollution of air in the city?	3	2 - 3
Does sore throat impair your ability to withstand heat or coldness?	2	1 - 3

IQR=Interquartile range.

safety, and moderately affected the endurance of air pollution in the city, heat or cold (Table 2). At the end of the questionnaire, the patients indicated quality of life on a scale from 0 to 100, where the median was 85 (IQR 80 - 85).

Follow-Up Examination Results

Data from the follow-up examination existed for 24 patients, while one patient did not provide answers for follow-up examination. All patients confirmed that they followed the instructions for use of the drug. Significant improvement was observed in pain, difficulty in swallowing, and feeling of the posterior pharyngeal wall swelling suggesting drug efficacy in non-infectious sore throat.

The median value of pain assessment at the first examination was 3 (IQR 1 - 4) compared to the follow-up examination where it was 0 (IQR 0 - 1), with a significant decrease after application of lysozyme-based therapy (P<0.001) (Figure 3). Six patients (24%) reported no pain at the first examination. At the follow-up examination none of the patients reported a worsening of the pain. Out of 18 patients who reported pain at the first examination, data for one patient was missing while all other patients reported a reduction in pain at follow-up. Nine patients who reported the presence of pain at the first examination, at the follow-up reported that the pain no longer existed.

The median value of the swallowing difficulty at the first examination was 3 (IQR 1 - 4) compared to the follow-up examination where it was 0 (IQR 0 - 1), with a significant decrease after applied therapy (P<0.001) (Figure 4). Four patients (16%) reported that there was no difficulty in swallowing at the first examination. Out of 21 patients who reported difficulty in swallowing at the first examination, at follow-up data was missing for one patient, none reported a deterioration in symptoms, one patient reported no improvement, while the other 19 reported an improvement. Ten patients who reported having difficulty swallowing at the first examination reported that difficulty no longer existed at the follow-up.

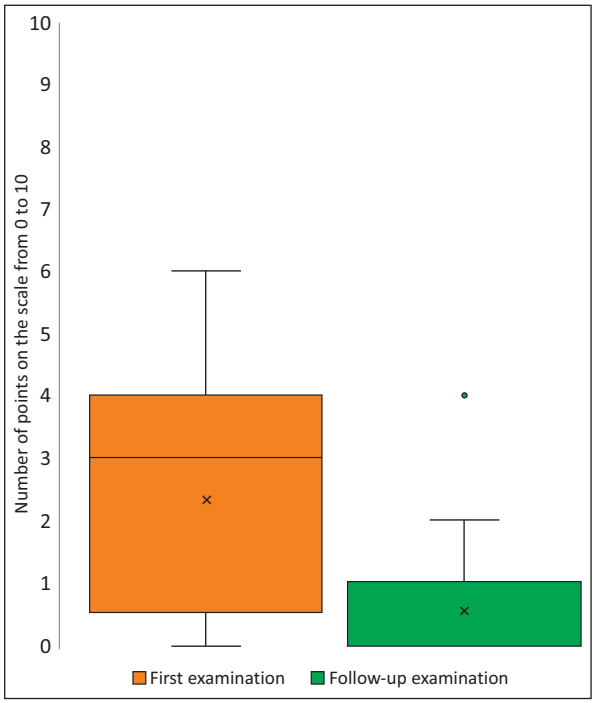


Figure 3. Assessment of pain at the first and follow-up examination. The median value was significantly decreased at the follow-up compared to the first examination (P<0.001). The middle line in the box represents median, the bottom line first quartile and the top line third quartile. The x in the box represents the mean and the whiskers extend to the minimum and maximum values, if no outliers are present or the first quartile minus 1.5 × interquartile range (IQR) and the third quartile plus 1.5 × IQR, if outliers are present. Outliers are represented by dots below or above whiskers.

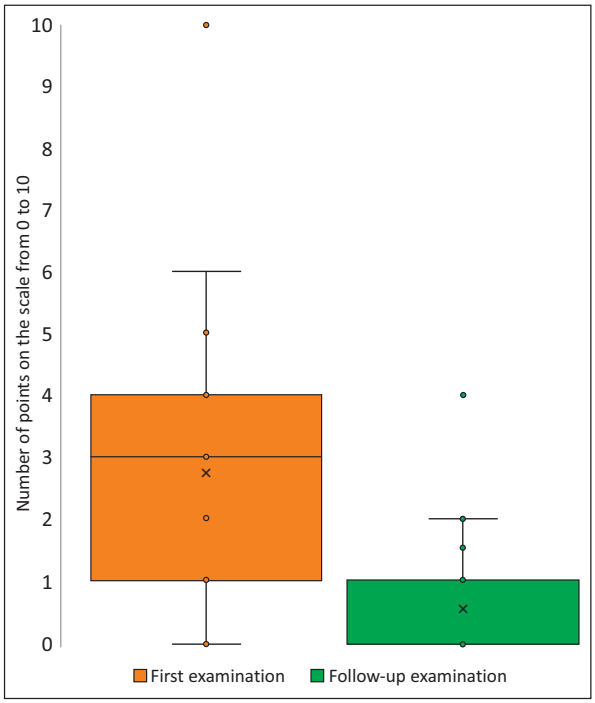


Figure 4. Assessment of swallowing difficulty at the first and follow-up examination. The median value was significantly decreased at the follow-up compared to the first examination (P<0.001). The middle line in the box represents median, the bottom line first quartile and the top line third quartile. The x in the box represents the mean and the whiskers extend to the minimum and maximum values, if no outliers are present or the first quartile minus 1.5 × interquartile range (IQR) and the third quartile plus 1.5 × IQR, if outliers are present. Outliers are represented by dots below or above whiskers.

The median value of the pharynx posterior wall swelling feeling at the first examination was 2 (IQR 1 - 4) compared to the follow-up examination where it was 0 (IQR 0 - 1), with a significant decrease after applied therapy (P<0.001) (Figure 5). Six patients (24%) reported at the first examination that there was no subjective feeling of swelling of the pharynx posterior wall. Out of 19 patients who reported swelling of the pharynx posterior wall at the first examination, at follow-up for one patient data was missing, none reported a worsening of the symptoms, two patients reported no improvement, while the other 16 reported improvement. Most patients reported an improvement of three points on a scale between 0 and 10. Ten patients who reported the presence of the pharynx posterior wall swelling at the first examination, reported at the follow-up that the feeling of swelling no longer existed.

22 patients (92%) at the follow-up examination confirmed that they would take the same medicine again due to the same problem. No drug adverse effects were reported in this study suggesting good safety of evaluated drug.

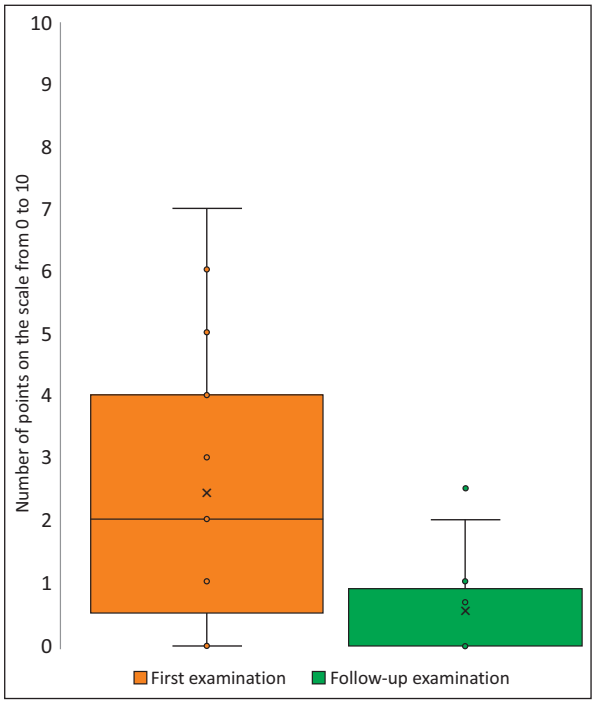


Figure 5. Assessment of the feeling of the pharynx posterior wall swelling at the first and follow-up examination. The median value was significantly decreased at the follow-up compared to the first examination (P<0.001). The middle line in the box represents median, the bottom line first quartile and the top line third quartile. The x in the box represents the mean and the whiskers extend to the minimum and maximum values, if no outliers are present or the first quartile minus 1.5 × interquartile range (IQR) and the third quartile plus 1.5 × IQR, if outliers are present. Outliers are represented by dots below or above whiskers.

Discussion

The results of this non-interventional pilot study indicated that lysozyme-based lozenges could have positive effects in relieving the symptoms of non-infectious sore throat in teachers. In the same time, the drug was found to be safe as there were no adverse effects reported in this study. This is the first study evaluating effects of lysozyme based medicines on non-infectious sore throat, but already known mechanism of action of this enzybiotic indicated that it could be beneficial for resolving non-infectious sore throat (12).

A significant improvement was observed in pain, difficulty swallowing, and subjective feeling of the posterior pharyngeal wall swelling after application of lysozyme-based therapy. 92% of the patients confirmed that they would take lysozyme-based lozenges again to treat the same problem. Beneficial effects of applied lozenges could be due to active substances lysozyme or pyridoxine but also due to effects of sucking lozenges.

Lysozyme's mechanisms of activity can be utilized to overcome inflammation in non-infectious sore throat. Lysozyme used in the pharmaceutical industry for the production of oral antiseptics and other lysozyme-based products, is derived from hen egg-white (16). This enzyme suppresses TNF-α and IL-6 in mouse macrophages (17), the main players in non-infectious sore throat inflammation (9, 10, 18, 19). Anti TNF-α activity of lysozyme was shown in human monocyte cells (20). Another active component of a drug used in this study, pyridoxine, is efficacious in treatment of diseases of oral cavity, gingivitis and glossitis. Pyridoxine has significant role in treatment of oral aphthae, pain and burning sensations in mouth (21). Lysozyme and pyridoxine combination is recommended for local treatment of painful aphthae as natural healing agent (22). In addition to active components activity, sucking lozenge could increase the salivary flow rate and lead to relieved symptoms in our patients. According to Tenovuo et al., a significant increase in salivary flow rate was observed immediately (2 - 4 min) after sucking the lozenge. Also, after the daily use of lozenge for one month the baseline flow rate was significantly elevated (23).

In addition to the proof-of-concept evaluation of lysozyme-based products in the treatment of non-infectious sore throat, this study investigated the characteristics of non-infectious sore throat in teachers, a high-risk profession for voice disorder as an occupational disease (24, 25). The most commonly studied group of professional voice users are teachers (1). In a study conducted in Brazil, non-infectious sore throat was reported by 63% of teachers (N=1651) and 36% of non-teachers (N=1614) (26). In a study conducted in Sweden, voice disorders were reported by 19.3% of teaching professionals (N=1173) (27). Behlau et al. reported

that the lifetime prevalence of non-infectious sore throat in teachers increases in the age group 30–39 years and persist across increasing ages. Also they found that women had higher prevalence of non-infectious sore throat across all ages (26). Non-infectious sore throat is usually manifested as vocal fatigue, hoarseness, throat pain or discomfort, weak voice, dryness, and lower pitch. Prevalence of symptoms in teachers after a long periods of voice usage in the classroom are vocal fatigue (52%), sore or dry throat (34%), vocal strain (29%), neck muscle tension (19%) and difficulty in projecting the voice (14%) (28).

We showed that non-infectious sore throat is a condition with moderate symptoms (medians for sore throat intensity and difficulty in swallowing were 3 on a scale between 1 and 5, most patients felt dry throat during the day, had normal tonsil size, none of the patients had an oropharyngeal enanthema and oropharyngeal mucosa was normal in 96% of patients). Non-infectious sore throat is generally less intensive when compared to infectious etiology (3, 4) and can be accompanied with pharyngitis as an isolated finding (10). Still, this condition can influence the quality of life in affected individuals (24) as we also found in our study (the median value of sore throat interference with work was 3 on a scale between 1 and 5, IQR 2 - 3). In a study with 1326 participants, 4.3% of them said that the voice disorder disabled them in their working tasks, and 7.2% were absent from work due to voice problems (25). In our study, the patients indicated the quality of life on a scale from 0 to 100, with the median 85 (IQR 80 - 85).

Risk factors for sore throat can be divided into different domains (physical, psychological, social, and environmental). In our study, physical, social, and environmental domains had medians of 2 on Likert scale from 1 (lowest) to 5 (highest) with exception of the question "Are you able to take care about yourself completely?" where scale from 1 (highest) to 5 (lowest) was used. Physical domain had the third quartile value 3, the highest among all domains (Table 2). Similarly, Kooijman et al. found that the highest risk factors for voice problems in teachers are within the physical and psycho-emotional domains. They found that appropriate voice training is of importance during teachers' education (29).

The most important risk factors for non-infectious sore throat development are female gender (24, 25, 29-32), air pollution (4), smoking, consuming alcoholic beverages (4, 22), and existing allergies (24, 32). We found that non-infectious sore throat impairs teachers' ability to withstand air pollution in the city. Additionally, a much higher proportion of patients in our study were women (80%). Some of the risk factors are diminished in teachers as a specific population. For instance, they are less likely to smoke or consume alcoholic beverages, compared to the general population (4, 24). In our study, only 1 in 25 patients (4%) consumed alcohol and 7 in 25 patients smoked (28%). In addition, only 2 in 25 patients (8%) had confirmed allergies. Despite the impact of non-infectious sore throat on life quality, there is a small number of patients looking for medical help due to this condition (4). Roy et al. found that 14.3% of teachers visited a doctor or speech-language pathologist for voice disorder (24), and in our study only 1 in 25 patients (4%) visited a doctor often due to the sore throat.

Limitation of Study

This was a pilot study involving 25 patients and only teachers were included. Only five patients (20%) were of male gender. Also, the study did not include control group, randomization and blinding. This was a "proof-of-concept" study and should be confirmed in controlled, blinded, randomized clinical trial on larger number of patients and in case of confirmation of drug efficacy and safety this drug should be considered as a treatment option for non-infectious sore throat.

Conclusion

This was a "proof-of-concept" study suggesting that lysozyme-based drugs in the form of lozenges might have positive effects in relieving the symptoms of non-infectious sore throat in teachers. At

the same time, the studied lozenges showed excellent tolerability and safety.

What Is Already Known on This Topic:

Non-infectious sore throat is a condition with significant effects on quality of life in persons using voice professionally, including teachers. Still, this condition is poorly recognized and therapeutic options are scarce. As inflammation is the underlying mechanism, compounds with anti-inflammatory and immunomodulatory effects are of great interest to be evaluated as therapeutics for non-infectious sore throat.

What This Study Adds:

This pilot study suggested positive effects of lysozyme based medication in treatment of non-infectious sore throat.

Authors' Contributions: Conception and design: SK, DŽH, MM, AŠ, JDŽJ, ATA, AS, AD, ZS and UG; Acquisition, analysis and interpretation of data: SK, DŽH and UG; Drafting the article: SK, MM, JDŽJ, ATA and UG; Revising it critically for important intellectual content: AŠ; Approved final version of the manuscript: SK, DŽH, MM, AŠ, JDŽJ, ATA, AS, AD, ZS and UG.

Conflict of Interest: Meliha Mehić, MD; Aziz Šukalo, MD; Jasna Džananović Jaganjac, PhD; Amna Tanović, MD; Una Glamočlija, PhD; disclose the following relationships – employees of Bosnalijek d.d., a pharmaceutical company producing lysozyme-based products. Bosnalijek d.d. had a role in the design of the study; in the collection, analyses, and interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Morawska J, Niebudek-Bogusz E. Risk factors and prevalence of voice disorders in different occupational groups – a review of literature. Otorynolaryngologia. 2017;16(3): 94-102.
2. Sanssené C, Bardi J, Welby-Gieusse M. Prevalence and Risk Factors of Voice Disorders in French Tour Guides. J Voice. 2020;34(6):911-7. doi: 10.1016/j.jvoice.2019.05.002.
3. Renner B, Mueller CA, Shephard A. Environmental and non-infectious factors in the aetiology of pharyngitis (sore throat). Inflamm Res. 2012;61(10):1041-52. doi: 10.1007/s00011-012-0540-9.
4. Addey D, Shephard A. Incidence, causes, severity and treatment of throat discomfort: a four-region online questionnaire survey. BMC Ear Nose Throat Disord. 2012;12:9. doi: 10.1186/1472-6815-12-9.
5. Cohen SM, Kim J, Roy N, Asche C, Courey M. Prevalence and causes of dysphonia in a large treatment-seeking population. Laryngoscope. 2012;122(2):343-8. doi: 10.1002/lary.22426.
6. Viswanatha GL, Thippeswamy AH, Rafiq M, Jagadeesh M, Baig MR, Suryakanth DA, et al. Novel experimental model of non-infectious pharyngitis in rats. J Pharmacol Toxicol Methods. 2014;69(2):189-95. doi: 10.1016/j.vascn.2013.12.001.
7. Uemura J, Nagpal R, Zerbinati N, Singh B, Marcellino M, Mohania D, et al. Effect of VBC-1814/7J, a polyphytocompound, on a non-infectious model of pharyngitis. Exp Ther Med. 2017;13(6):3075-80. doi: 10.3892/etm.2017.4332.
8. Viswanatha GL, Rafiq M, Thippeswamy AHM, Yuvaraj HC, Kavya KJ, Baig MR, et al. Ameliorative effect of Koflet formulations against pyridine-induced pharyngitis in rats. Toxicol Rep. 2014;1:293-9. doi: 10.1016/j.toxrep.2014.05.003.
9. Renner B, Ahne G, Grosan E, Kettenmann B, Kobal G, Shephard A. Tonic stimulation of the pharyngeal mucosa causes pain and a reversible increase of inflammatory mediators. Inflamm Res. 2013;62(12):1045-51. doi: 10.1007/s00011-013-0663-7.
10. Muhrer JC. Diagnostic considerations in the evaluation and treatment of sore throat. Nurse Pract. 1991;16(9):33-8, 41, 45.
11. Hauber HP, Rüller S, Müller E, Hansen E, Zabel P. Pharyngeal lavage lymphocytosis in patients with obstructive sleep apnea: a preliminary observation. PLoS One. 2011;6(1):e16277. doi: 10.1371/journal.pone.0016277.
12. Cartel F, Cartei G, Ceschia V, Pacor S, Sava G. Recovery of Lymphocyte CD4+: CD8+ Ratio in Patients Treated with Lysozyme. Drug Invest. 2012;4(1):51-7.
13. Glamočlija U, Mehić M, Šukalo A, Tanović Avdić A, Džananović Jaganjac J. Lysozyme in the treatment of non-infectious sore throat. Bosn J Basic Med Sci. 2020;20(2):281-2. doi: 10.17305/bjbms.2019.4619.
14. Catic T, Kapo B, Pintol Z, Skopljak A, Cengic A, Gojak R, et al. An Instrument for Rating Quality of Life Related to Sore Throat in Patients Suffering from Acute Pharyngitis or Tonsillitis. Mater Sociomed. 2018;30(1):43-8. doi: 10.5455/msm.2018.30.43-48.
15. Lysobact 30 compressed lozenge [in Bosnian]. Patient Information Leaflet. 04-07.3-2-3062/17 dating July 20, 2017.
16. Leśnierowski G, Cegielska-Radziejewska R. Potential possibilities of production, modification and practical application of lysozyme. Acta Sci Pol Technol Aliment. 2012;11(3):223-30.
17. Tagashira A, Nishi K, Matsumoto S, Sugahara T. Anti-inflammatory effect of lysozyme from hen egg white on mouse peritoneal macrophages. Cytotechnology. 2018;70(3):929-38. doi: 10.1007/s10616-017-0184-2.
18. Manzano-León N, Serrano-Lomelin J, Sánchez BN, Quintana-Belmares R, Vega E, Vázquez-López I, et al. TNFα and IL-6 Responses to Particulate Matter in Vitro: Variation According to PM Size, Season, and Polycyclic

Aromatic Hydrocarbon and Soil Content. Environ Health Perspect. 2016;124(4):406-12. doi: 10.1289/ehp.1409287.

19. van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T, et al. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). Am J Respir Crit Care Med. 2001;164(5):826-30. doi: 10.1164/ajrccm.164.5.2010160.
20. Bergamo A, Gerdol M, Pallavicini A, Greco S, Schepens I, Hamelin R, et al. Lysozyme-Induced Transcriptional Regulation of TNF-α Pathway Genes in Cells of the Monocyte Lineage. Int J Mol Sci. 2019;20(21):5502. doi: 10.3390/ijms20215502.
21. Topić B, Malić M, Arifhodžić F, Živojnović V. The use of Lyso-B in stomatitis aphtose treatment. Stomatolo vjesn. 1979;8:129-33.
22. Kone-Paut I, Barete S, Bodaghi B, Deiva K, Desbois AC, Galeotti C, et al. French recommendations for the management of Behçet's disease. Orphanet J Rare Dis. 2021;16(Suppl 1):352. doi: 10.1186/s13023-020-01620-4.
23. Tenovuo J, Hurme T, Ahola A, Svedberg C, Ostela I, Lenander-Lumikari M, et al. Release of cariostatic agents from a new buffering fluoride- and xylitol-containing lozenge to human whole saliva in vivo. J Oral Rehabil. 1997;24(5):325-31. doi: 10.1046/j.1365-2842.1997.00519.x.
24. Roy N, Merrill RM, Thibeault S, Parsa RA, Gray SD, Smith EM. Prevalence of voice disorders in teachers and the general population. J Speech Lang Hear Res. 2004;47(2):281-93. doi: 10.1044/1092-4388(2004/023).
25. Roy N, Merrill RM, Gray SD, Smith EM. Voice disorders in the general population: prevalence, risk factors, and occupational impact. Laryngoscope. 2005;115(11):1988-95. doi: 10.1097/01.mlg.0000179174.32345.41.
26. Behlau M, Zambon F, Guerrieri AC, Roy N. Epidemiology of voice disorders in teachers and nonteachers in Brazil: prevalence and adverse effects. J Voice. 2012;26(5):665.e9-18. doi: 10.1016/j.jvoice.2011.09.010.
27. Lyberg-Åhlander V, Rydell R, Fredlund P, et al. Prevalence of Voice Disorders in the General Population, Based on the Stockholm Public Health Cohort. J Voice Off J Voice Found. 2019;33:900–905. doi: 10.1016/j.jvoice.2018.07.007
28. Devadas U, Bellur R, Maruthy S. Prevalence and Risk Factors of Voice Problems Among Primary School Teachers in India. J Voice. 2017;31(1):117.e1-10. doi: 10.1016/j.jvoice.2016.03.006.
29. Kooijman PG, de Jong FI, Thomas G, Huinck W, Donders R, Graamans K, et al. Risk factors for voice problems in teachers. Folia Phoniatr Logop. 2006;58(3):159-74. doi: 10.1159/000091730.
30. Alva A, Machado M, Bhojwani K, Sreedharan S. Study of Risk Factors for Development of Voice Disorders and its Impact on the Quality of Life of School Teachers in Mangalore, India. J Clin Diagn Res. 2017;11(1):MC01-5. doi: 10.7860/JCDR/2017/17313.9234.
31. Zabret M, Hočevar Boltežar I, Šereg Bahar M. The Importance of The Occupational Vocal Load for The Occurence and Treatment of Organic Voice Disorders. Zdr Varst. 2018;57(1):17-24. doi: 10.2478/sjph-2018-0003.
32. Trinite B. Epidemiology of Voice Disorders in Latvian School Teachers. J Voice. 2017;31(4):508.e1-9. doi: 10.1016/j.jvoice.2016.10.014.

Appendix

Translated test list used for the study

Name of the study:

Non-interventional Pilot Study Evaluating the Efficacy and Safety of Lysozyme-based Therapy in Patients with Non-infectious Sore Throat

Patients' initials:

Number of patient:

GENERAL QUESTIONNAIRE

Gender: Male Female

Age: 18 - 30 30 - 50 50 - 65

Length of service:
- ☐ < 5 years
- ☐ 5 do 10 years
- ☐ 10 do 20 years
- ☐ 20 do 30 years
- ☐ > 30 years

Working place: ☐ Elementary school ☐ High school ☐ Music school

How often do you use your voice without a break during the day?

☐ Less than 30 minutes ☐ 30 - 60 minutes ☐ More than 60 minutes

Do you smoke? YES NO

Do you consume alcohol? YES NO

If the answer is YES, state the average number of alcoholic beverages during:

Week ____ Month ____ Year ____

Do you use air conditioning at work or at home? YES NO

Do you have a confirmed allergy? YES NO

If the answer is YES, specify the type of allergy _____

Do you feel hoarse during the day? YES NO

If the answer is YES, state how often you feel hoarse _____

Do you feel a dry throat during the day? YES NO

If the answer is YES, state how often you feel a sore throat _____

Do you drink fluids often during working hours? YES NO

If the answer is YES, state how much fluid you drink during the day (L) _____

Do you have a bad breath? YES NO

Does a sore throat make you nervous? YES NO

Do sore throat decrease when you keep quiet? YES NO

Do you visit the doctor often because of a sore throat? YES NO

If the answer is YES, how often have you visited a doctor in the last year for a sore throat? _____

Do you use any of the preparations for throat? YES NO

If the answer is YES, specify the type of preparation: _____

FIRST EXAMINATION

PHYSICAL EXAMINATION (assessment of tonsillopharyngitis)

Oropharyngeal mucosa color

Normal/ Pink or slightly red ☐ Red ☐ Very red ☐

The size of the tonsils

Normal/ Not increased ☐ Slightly increased ☐ Moderately increased ☐ Very increased ☐

Oropharyngeal enanthema (vesicles, petechiae, exudate)

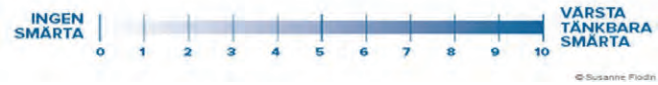
Not present ☐ Few ☐ Many ☐ Very much ☐

ASSESSMENT OF PAIN, DIFFICULTY IN SWALLOWING AND SUBJECTIVE FEELING OF THE POSTERIOR PHARYNGEAL WALL SWELLING

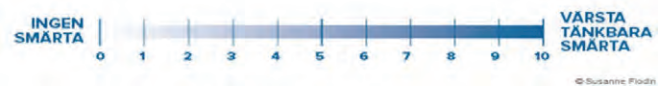
Instructions: Mark the strength/intensity with a cross

Pain

Difficulty swallowing



Subjective feeling of swelling of the posterior wall of the pharynx



** During the first examination, the Questionnaire for Measuring the Quality of Life Related to Sore Throat is also filled in (attached to the test list)*

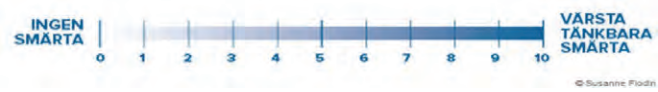
FOLLOW-UP EXAMINATION

Instructions: Mark the strength/intensity with a cross

Pain

Difficulty swallowing

Subjective feeling of swelling of the posterior wall of the pharynx



Have you followed the instructions for use of the medicine?	YES	NO
Would you take same medicine again because of the same ailment?	YES	NO

Original Clinical Research
Acta Medica Academica 2022;51(1):14-20
DOI: 10.5644/ama2006-124.365

Femoral 3-in-1 Nerve Block for Total Knee Replacement, an Analgesic Approach Not to Be Neglected. Single Center Experience and Literature Review

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Received: 30 January 2022; **Accepted:** 20 April 2022

Abstract

Objectives. Total Knee Replacement Surgery (TKR) is one of the most common elective orthopedic operations. Postoperative pain after total knee replacement, remains a challenge. In this retrospective observational study, we evaluated the effectiveness of 3-in-1 nerve block in patients after total knee arthroplasty compared to standard opioid treatment, and we state the reasons why this approach should still be considered. **Methods.** To evaluate the effectiveness of the 3-in-1 nerve block, we assessed the acute pain service archive and compared the values of the visual analog scale, by separating patients into two groups according to the analgesic regimen they received as per local protocols. In group A, patients received 0.25% bupivacaine through a 3 in 1 block catheter and additional meperidine IM if needed, while in group B they received meperidine every six hours. **Results.** Our analysis showed the statistically significant better effectiveness of 3-in-1 nerve block with bupivacaine administration in postoperative TKR pain control compared to repeated administration of meperidine. **Conclusion.** The results of our study suggest that 3-in-1 nerve block with bupivacaine is an option that must always be considered in order to alleviate post-operative pain after TKR.

Key Words: Postoperative Pain ▪ 3-in-1 Nerve Block ▪ Femoral Nerve Block ▪ Total Knee Replacement.

Introduction

Steadily increasing life expectancy makes the greater part of the population susceptible to degenerative diseases, with osteoarthritis being one of the most common (1). Due to both obesity and trauma, lower limbs are commonly affected, with an incidence of 41% in the knee and 19% in the hips. Several studies have shown that knee osteoarthritis severely affects the quality of life, causing physical disability and increased mortality. Thankfully, when this condition cannot be managed with conservative approaches, total knee replacement (TKR) surgery has become the 'salvation' treatment (2). TKR is currently a routinely performed operation of medium morbidity and low mortality because of the advanced surgical approaches used, the improved quality of materials, and the enhanced anesthesiology strategies deployed (3). However, management of postoperative pain after total knee replacement still remains a challenge. Sixty percent of these patients have severe postoperative pain and 30% refer to moderate levels of postoperative pain (4).

Insufficient postoperative analgesia has a strong negative impact on patients' health by increasing the risk of cardiovascular incidents, while impeding early mobilization of the knee. Delayed or reduced mobilization carries the imminent consequences of dysfunction, ligament contractions and muscle atrophy, which may retard or in the worst cases, hinder the restitution of the knee function.

In order to prevent this, the majority of postoperative programs for remobilization start within the first 24 hours, or in some cases immediately after the operation (PACU), and include physiotherapy and application of continuous passive mobilization (5). Despite the development of a wide spectrum of rehabilitation protocols the main obstacle for their application is pain. Several analgesic techniques have been tested over the years to alleviate pain sufficiently. None of them has yet proven superior enough to become a gold standard (6). So, in many centers, including ours, traditional, well-established, scientifically recommended and effective approaches are still used.

In this study, we aimed to evaluate the current role of the femoral "3 in 1" nerve block with a catheter, in patients who had undergone TKR.

Methods

Study Design and Population

The study was conducted at the University Hospital of Heraklion by analyzing data acquired in the first semester of 2015. As it is a retrospective study assessing acute pain service from medical records no approval was required from our institutional review board. According to local protocols, patients receive either opioids systemically, or a neural block is performed in order to achieve postoperative analgesia. The regimen that is selected each time depends on both the patient's and the anesthetist's preferences. In this retrospective observational study, we included 42 patients who underwent total knee replacement under spinal anesthesia, and had similar demographics, minor or no comorbidities, an ASA physical status score of 1-2, and aged between 50 and 79 years old. Patients with severe comorbidities or patients that received other analgesic regimens (multiple opioids, analgesic adjuvants) or perioperative sedation were excluded from the analysis. In order to minimize bias attributed to surgical technique-induced pain, we only included patients operated by the same surgeon. Analysis was performed by allocating patients to two groups on the basis of the postoperative analgesic approach they received, which was jointly decided after discussion between the patient and the anesthetist. In group A, patients had a femoral 3-in-1 block with a catheter in place, and pain was alleviated with repeated boluses of local anaesthetic with opioids as rescue therapy, while in group B (control) the pain was managed with administration of opioids in regular divided doses.

Implementation Process

According to local protocols, Group A was treated with repeated doses of 40ml bupivacaine 0.25% via a "3-in-1 catheter" and additionally meperidine 50 mg (IM) as a rescue analgesic if no adequate pain control was achieved with bupivacaine alone. In group B repeated meperidine boluses of 50mg (IM) were given every 6 hours.

To perform the "3-in-1" block, there is a standardized approach in our department that is applied to all patients in the same way. Specifically, patients were seated in a comfortable position and their leg was rotated 15 degrees outwards. The inguinal area was sterilized and local infiltration of the area with 3 ml lignocaine 2% was performed. Then a 9.5 G (pajunk) needle was inserted, 2.5 cm inferior of the inguinal ligament and 1.0 cm lateral of the femoral artery. The femoral nerve was located with the use of a nerve stimulator (stimuplex S Braun). The exact point was identified where contraction of the quadriceps muscle was achieved with the lowest intensity of stimulus (current of 0.5 mA in 0.1 ms). Then, with a modified seldinger technique (catheter-through the needle) the catheter (pajunk-plexolong) was advanced for a distance of about 10cm into the neural sheath. After verification of the position of the catheter, with no blood to be aspirated, an antibacterial filter was connected. After this, 40 ml of 0.25% bupivacaine were injected, prior to initiation of anesthesia. All patients received spinal anaesthesia, with hyperbaric bupivacaine 0.5% w/v at L3 - L4 or L4 - L5 at a dose of approximately 12 mg depending on the

patient's height, and one dose of Tenoxicam 20 mg IV was administered.

Data Collection

We assessed the acute pain service archive in order to retrieve data for patients who fulfilled the criteria mentioned above according to their preoperative assessment sheet and intraoperative diagram. Records included pain scores from PACU (1st hour), 6 and 9 hours postoperatively, and thereafter every 12 hours until 72 hours postoperatively, with the use of the 100mm Visual Analogue Scale (VAS). Furthermore, the time when the patient was able to walk was noted. Other vital parameters were also recorded (Ramsay score, heart rate, respiratory rate, blood pressure - Data not shown). The total dose of meperidine administered IM (in both groups) was calculated. Side effects related to analgesia were also noted in the records (nausea, vomiting, intestinal paralysis, respiratory depression).

Statistical Analysis

Data were analyzed with SPSS Version 23 statistical software. We performed an unpaired t-test to compare meperdine dose and boluses, as assumptions were satisfied, and a Chi-Square test for sex. Finally we used the Mann-Whitney U test for VAS Scores and the rest of the variables. A two-sided significance level of P=0.05 was used for all tests.

Results

Forty-two patients, 15 men and 27 women, median age 67.25, were analyzed in the study. There were no significant differences regarding the patients' demographics between the two groups. Group B patients needed a significantly higher mean total dose of meperidine: Group A = 12.5 mg vs. Group B = 520 mg. Thus, the majority of patients in the study group were sufficiently covered by the 3 in 1 block alone, with no need for extra analgesics. Only three patients in the study group needed additional meperidine. As far as the length of hospital stay is concerned, it was prolonged by almost a day in group B (control), further increasing the costs. Another parameter evaluated was 'Days to Walk', which refers to the period from the surgical procedure to the moment that the patient was able to stand up with minor support and walk a distance of a few meters. We noticed that this period was significantly reduced in Group A, by almost a day (P<0.05), making the patient independent earlier, as well as further diminishing problems related to prolonged bed stay (Table 1).

Regarding the intensity of pain, VAS scores both at rest and during movement were significantly lower in group A in comparison to group B, except for the values at 48h where no statistical significance was shown (Tables 2 and 3).

There were no significant variations in the vital parameters and no opioid side effects were reported in either patient group.

Table 1. Study Population Characteristics and Main Study Variables

Characteristics	Group A (3 in 1 block)		Group B (Meperidine)		P values
Number of patients	21		21		
Gender (N)	Male	Female	Male	Female	0.747
	8	13	7	14	
Mean Age	67± 5 (median 65.8)		69 ± 4 (median 68.7)		0.510
Number of meperidine boluses demanded	0.25 ± 0.58		10.4 ± 1.1		0.001
Total dose of meperidine (mg)	12.5 ± 29		520 ± 55		0.001
Days to walk	1.3 ± 0.6		2.2 ± 0.7		0.001
Blood Loss (ml)	390 ± 120		480 ± 90		-
Hospital Stay (days)	7.4 ± 0.6		8.2 ± 0.8		0.002

The Chi-square test was used for sex comparison, the T-test for meperdine boluses and dose, while the Mann-Whittney U test was used for all other variables. No P value is provided for blood loss as there are values missing.

Table 2. Mean Values of VAS Scores Movement

Hours post OP (h)	Group A (3 in 1 block)	Group B (Meperidine)	P values
	VAS Score Movement		
1	0.625±0.25	17±0.9	0.001
6	0.0±0.0	32.5±0.44	0.001
9	1.25±0.50	21.25±0.34	0.001
24	28.12±0.98	32.5±0.44	0.012
48	38.75±4.31	30.62±0.25	0.275
72	25.62±0.51	30.62±0.57	0.003

The mean values of VAS scores during movement in both groups at different times, as shown on the vertical axis. Note that at 48 h the scores of the 3-in-1 group are a higher than the control. The Mann-Whitney U test was performed for data analysis.

Table 3. Mean Values of VAS Scores at Rest

Hours Post OP (h)	Group A (3 in 1 block)	Group B (Meperidine)	P values
	VAS Score at rest		
1	0.0 ± 0.0	12.50±0.44	0.001
6	0.0 ± 0.0	21.25±0.50	0.001
9	0.0 ± 0.0	10.0±0.63	0.001
24	13.12±0.79	21.25±0.34	0.001
48	13.75±0.80	19.37±0.57	0.015
72	11.25±0.88	21.87±0.75	0.001

The mean values of VAS scores at rest in both groups at different times, as shown on the vertical axis. The Mann-Whitney U test was performed for data analysis.

Discussion

In the present study, we compared the impact of two different guideline-recommended (7) methods of management of postoperative pain after Total Knee Replacement surgery. However, numerous other techniques are usually employed such as: i) demand-adapted intravenous analgesia (PCA), ii) epidural analgesia with opioids, local anesthetics or both, iii) lumbar nerve block, iv) standard analgesia per os or intramuscularly v) peripheral nerve blocks vi) local infiltration (8, 9). Of the most commonly used, both systemic opioids with conventional PCA and NSAIDs have several side effects and result in inadequate pain control, making the initiation of early intense physical therapy impossible. Respectively, epidural analgesia with continuous infusion of opioids and/or local anesthetics may result in bilateral motor blockade, and side effects such as nausea, urinary retention, pruritus and respiratory depression. This is the reason why the literature has mainly focused on peripheral nerve blockade. Working in this direction, we studied the efficacy of the 3-in-1 block, and we showed clearly that it provides superior analgesia compared to systemic opioid administration. The "3-in-1" nerve block has been proven to be an effective form of pain control after open knee surgery, with local anesthetics injected into the nerve sheath of the femoral, the femoral lateral cutaneous and the obturator nerve (4, 10-14). Consequently, the anatomical distribution of these nerves may explain our finding at 48h (the moment of the most intense mobilization) as patients mentioned pain in the posterior area of the knee, as the afferent fibers travel through the sciatic nerve branches.

Several variants of the 3-in-1 approach have been reported over the years in the literature, either using a single shot or continuous infusion. This technique, which was also adopted in our study, was first described by Winnie et al. (15) in 1973 as a superior alternative to femoral nerve block. The rationale behind this method was to substitute unilateral epidural analgesia. This is possible owing to the anatomical enclosure of the femoral, lateral cutaneous and obturator nerves in a common sheath, beginning almost right after the merger of the nerve root. In order to achieve this type of analgesia with a single shot technique, higher volumes of local anesthetics are used (approximately 40 ml compared to 15-20ml) with simultaneous distal pressure on the nerve sheath to achieve central dispersion of the anesthetic. On the other hand, when a catheter is introduced, it is directed cephalad and not distally as in continuous "femoral nerve block" (FNB).

The efficiency of the 3-in-1 femoral nerve block using different local anesthetics as well as different concentrations, for treating severe postoperative pain after TKR was tested by Ng et al. (11) The study showed no statistically significant difference between ropivacaine and bupivacaine groups in

terms of equianalgesic doses. Furthermore, no advantage was shown using a higher concentration of ropivacaine (0.25% vs 0.5%). The results of this study are in agreement with our results, confirming the effectiveness of the 3-in-1 nerve block in pain control after TKR.

To further evaluate the efficiency of this method, other researchers also compared it with intravenously injected opioids or regional techniques. Specifically, Ozen et al. (12) tested the use of a single-shot 3-in-1 femoral nerve block preoperatively in patients undergoing total knee replacement, and found a significant decrease in postoperative morphine consumption. The 3-in-1 nerve block group that received 40ml 0.375% ropivacaine, experienced no pain eight hours after surgery in the recovery room, and morphine requirements were significantly lower 12, 18, 24, 48 hr after TKR ($P<0.001$), which also decreased the occurrence of complications. The second group received only 2 mg of morphine as a loading dose 30 minutes before the end of surgery, and experienced pain of medium severity in the immediate postoperative period, which was sufficiently controlled (VAS score ≤ 30) with supplementary analgesia within the first hour in the post-anesthesia care unit (PACU).

In another fundamental study in the field, F J Singelyn et al. (10) compared a continuous 3-in-1 block with standard morphine PCA and epidural anesthesia. Their results also suggested that both epidural and nerve blocks provide superior analgesia after TKR, but the epidural was associated with four times more complications than continuous femoral nerve block. Complications such as urinary retention, arterial hypotension, and motor block are quite common. Problems related to epidural catheters must also be considered when choosing an analgesic regimen, for example, the challenging management of anticoagulants as prophylaxis for deep vein thrombosis, and the possibility of the failure of a central neuraxial block (16). The absence of such limitations with the 3-in-1 block highlights the importance of having this technique in our therapeutic armoire.

Similar results were also shown by Theodosiadis et al. (17) regarding anesthetic and analgesic effects, with ropivacaine having a significantly faster onset time. The researchers in this study suggest that not only the onset time but also the duration of the blockade, and the safety of the injected drug should be considered in order to select the optimal substance for the 3-in-1 block. They also mention that, despite the good safety profile of this method, complications may also exist, such as incomplete nerve blockade, direct nerve trauma, with potential quadricep wasting, local hematoma, ischemic injury, and infection, or even falls (18), suggesting the need for frequent reassessment of patients, especially of their motor function (17). On the other hand, adductor canal block permits early ambulation as it does not affect motor function. The continuous infusion variant in particular has a similar safety and efficacy profile to continuous femoral nerve block (19, 20). This explains why this approach is currently commonly used for TKA pain management and has become the subject of recent studies published in the literature (19, 20). However, we found no studies directly comparing continuous adductor canal block to the continuous 3-in-1 nerve block variant.

It is also important to mention that it is quite ambiguous whether the 3-in-1 block is actually a different modality (21) to the well-known femoral nerve block as far as the clinical effect is concerned. Despite the well-described steps of this variation and verification via magnetic resonance imaging, showing that there is a different dispersion of the local anesthetic centrally, in a limited number of patients this technique failed to achieve sufficient levels of analgesia, which was possibly attributed to anatomical (22) or technical factors. Capdevilla et al. (23) showed that only 40% of catheter tips were in the 'ideal' position, but with no correlation with the final analgesic effect. Due to these phenomena, in the literature 3-in-1 block is included within the wider term of FNB, and some researchers even suggest abandoning the term (24). This could be a limitation both to our study and others investigating the 3-in-1 variation of femoral nerve block as far as comparison and interpretation of results is concerned.

Finally, the recent systematic reviews and metanalyses by Karlsen et al. (13) and Chan EY et al. (14) document the efficacy and safety of analgesic interventions after total knee replacement, demonstrating that there is no optimal strategy to manage postoperative pain after TKR. The acceptable level of pain presented great variability in trials, something to be expected when taking the subjectivity of pain perception into account. In some of them, no basic analgesic substance was used and high pain scores were accepted, whereas in others several pain management interventions were tested, e.g. FNB etc. The differences led to considerable sensitivity variance between trials, which was probably caused by the several factors that differed between the study groups, making it almost impossible to interpret and compare results safely. Despite the mediocre level of bias between studies included in the metanalysis mentioned above, continuous femoral nerve block (3-in-1 included) achieved a mean opioid-sparing effect that was similar to the one-shot technique, and both were somewhat superior to placebo/standard opioid analgesia according to the results after the first 24 h. Furthermore, the continuous nerve block variant showed its benefit at 24h with lower VAS scores, especially during movement, compared to the one-shot. Such benefits would be even more prominent if the analysis also included the subsequent postoperative days, as suggested by our study.

Limitations of Study

Both the retrospective analysis method and the small number of patients included, are limitations of this study.

Conclusion

The efficiency of 3-in-1 nerve block as a postoperative pain managment intervention in patients after TKR and its superior analgesic effects are further verified in our study, justifying the inclusion of this peripheral nerve blockade approach, in current pain management guidelines. However, further double-blind, randomized, multi-centered studies are required to elucidate the labyrinthine pathway of pain management in such cases. Future studies should focus on showing the most effective femoral nerve block variation, but also evaluating the use of adjuvants in 3-in-1 block in order to optimize our practice.

What Is Already Known on This Topic:
Several systematic reviews and metanalyses show the superiority of regional techniques over opioids and IV analgesics in the management of postoperative pain after TKR, with most studies being in favor of neural blocks due to their safety profile. Simultaneously, several problems are mentioned in the same studies with failure of or insufficient analgesia when a single nerve is targeted, thus reintroducing the need for systematic analgesia as a rescue solution, or performing multiple blocks. The variability of study designs with several comparisons between different analgesic regimens in each study does not provide us high levels of evidence so the guidelines still suggest all approaches (IV analgesics, nerve blocks and neuraxial anesthesia) as preferable for pain management.

What This Study Adds:
This study further verifies the superiority of nerve blocks and especially of the 3-in-1 block variant which simultaneously targets more than one nerve, providing superior levels of analgesia, with mean VAS scores of less than 2 and 3 at rest and during movement, respectively. These findings suggest that the 3-in-1 block variant should not be abandoned. Furthermore, in the discussion part, we summarize several reasons that explain the variability of the success rates of this technique among patients, which may have led to misinterpretation of results in past studies and which possibly explain the inhomogeneity of pain management in everyday clinical practice.

Authors' Contributions: Conception and design: VK, NS and KBa; Acquisition, analysis and interpretation of data: VK and KBr; Drafting the article: VK, KBr, ID, IS and KBa; Revising it critically for important intellectual content: VK, AP and KBa; Approved final version of the manuscript: VK, KBr, NS, AP, ID, IS and KBa.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Di Nicola V. Degenerative osteoarthritis a reversible chronic disease. Regen Ther. 2020;15:149-60. doi: 10.1016/j.reth.2020.07.007.
2. Wood AM, Brock TM, Heil K, Holmes R, Weusten A. A Review on the Management of Hip and Knee Osteoarthritis. Int J Chronic Dis. 2013;2013:845015. doi: 10.1155/2013/845015.
3. Turnbull ZA, Sastow D, Giambrone GP, Tedore T. Anesthesia for the patient undergoing total knee replacement:

current status and future prospects. Local Reg Anesth. 2017;10:1-7. doi: 10.2147/LRA.S101373.

4. Edwards ND, Wright EM. Continuous low-dose 3-in-1 nerve blockade for postoperative pain relief after total knee replacement. Anesth Analg. 1992;75(2):265-7. doi: 10.1213/00000539-199208000-00020.
5. Pagnotta G, Rich E, Eckardt P, Lavin P, Burriesci R. The Effect of a Rapid Rehabilitation Program on Patients Undergoing Unilateral Total Knee Arthroplasty. Orthop Nurs. 2017;36(2):112-21. doi: 10.1097/NOR.0000000000000325..
6. Krishna Prasad GV. Post-operative analgesia techniques after total knee arthroplasty: A narrative review. Saudi J Anaesth. 2020;14(1):85-90. doi: 10.4103/sja.SJA_494_19.
7. Fischer HB, Simanski CJ, Sharp C, Bonnet F, Camu F, Neugebauer EA, et al. A procedure-specific systematic review and consensus recommendations for postoperative analgesia following total knee arthroplasty. Anaesthesia. 2008;63(10):1105-23. doi: 10.1111/j.1365-2044.2008.05565.x.
8. Schultz P, Anker-Møller E, Dahl JB, Christensen EF, Spangsberg N, Faunø P. Postoperative pain treatment after open knee surgery: continuous lumbar plexus block with bupivacaine versus epidural morphine. Reg Anesth. 1991;16(1):34-7.
9. Paglia A, Goderecci R, Ciprietti N, Lagorio M, Necozione S, Calvisi V. Pain management after total knee arthroplasty: A prospective randomized study. J Clin Orthop Trauma. 2020;11(1):113-7. doi: 10.1016/j.jcot.2018.12.005.
10. Singelyn FJ, Deyaert M, Joris D, Pendeville E, Gouverneur JM. Effects of intravenous patient-controlled analgesia with morphine, continuous epidural analgesia, and continuous three-in-one block on postoperative pain and knee rehabilitation after unilateral total knee arthroplasty. Anesth Analg. 1998;87(1):88-92. doi: 10.1097/00000539-199807000-00019.
11. Ng HP, Cheong KF, Lim A, Lim J, Puhaindran ME. Intraoperative single-shot "3-in-1" femoral nerve block with ropivacaine 0.25%, ropivacaine 0.5% or bupivacaine 0.25% provides comparable 48-hr analgesia after unilateral total knee replacement. Can J Anaesth. 2001;48(11):1102-8. doi: 10.1007/BF03020376.
12. Ozen M, Inan N, Tümer F, Uyar A, Baltaci B. The effect of 3-in-1 femoral nerve block with ropivacaine 0.375% on postoperative morphine consumption in elderly patients after total knee replacement surgery. Agri. 2006;18(4):44-50.
13. Karlsen AP, Wetterslev M, Hansen SE, Hansen MS, Mathiesen O, Dahl JB. Postoperative pain treatment after total knee arthroplasty: A systematic review. PLoS One. 2017;12(3):e0173107. doi: 10.1371/journal.pone.0173107.
14. Chan EY, Fransen M, Parker DA, Assam PN, Chua N. Femoral nerve blocks for acute postoperative pain after knee replacement surgery. Cochrane Database Syst Rev. 2014;2014(5):CD009941. doi: 10.1002/14651858.CD009941.pub2.
15. Winnie AP, Ramamurthy S, Durrani Z. The inguinal paravascular technic of lumbar plexus anesthesia: the "3-in-1 block". Anesth Analg. 1973;52(6):989-96.
16. Iwata T, Lakshman S, Singh A, Yufa M, Claudio R, Hadzić A. Peripheral nerve blocks for perioperative management of patients having orthopedic surgery or trauma of the lower extremity. Bosn J Basic Med Sci. 2005;5(2):5-19. doi: 10.17305/bjbms.2005.3278.
17. Theodosiadis P, Sachinis N, Goroszeniuk T, Grosomanidis V, Chalidis B. Ropivacaine versus bupivacaine for 3-in-1 block during total knee arthroplasty. J Orthop Surg (Hong Kong). 2013;21(3):300-4. doi: 10.1177/230949901302100307.
18. Atkinson HD, Hamid I, Gupte CM, Russell RC, Handy JM. Postoperative fall after the use of the 3-in-1 femoral nerve block for knee surgery: a report of four cases. J Orthop Surg (Hong Kong). 2008;16(3):381-4. doi: 10.1177/230949900801600324.
19. Zhang Z, Wang Y, Liu Y. Effectiveness of continuous adductor canal block versus continuous femoral nerve block in patients with total knee arthroplasty: A PRISMA guided systematic review and meta-analysis. Medicine (Baltimore). 2019;98(48):e18056. doi: 10.1097/MD.0000000000018056.
20. Sun C, Zhang X, Song F, Zhao Z, Du R, Wu S, Ma Q, Cai X. Is continuous catheter adductor canal block better than single-shot canal adductor canal block in primary total knee arthroplasty?: A GRADE analysis of the evidence through a systematic review and meta-analysis. Medicine (Baltimore). 2020;99(20):e20320. doi: 10.1097/MD.0000000000020320.
21. Dupré LJ. Three-in-one block or femoral nerve block. What should be done and how? [in French]. Ann Fr Anesth Reanim. 1996;15(7):1099-101. doi: 10.1016/s0750-7658(96)89483-4.
22. Ritter JW. Femoral nerve "sheath" for inguinal paravascular lumbar plexus block is not found in human cadavers. J Clin Anesth. 1995;7(6):470-3. doi: 10.1016/0952-8180(95)00055-m.
23. Capdevila X, Biboulet P, Morau D, Bernard N, Deschodt J, Lopez S, et al. Continuous three-in-one block for postoperative pain after lower limb orthopedic surgery: where do the catheters go? Anesth Analg. 2002;94(4):1001-6, table of contents. doi: 10.1097/00000539-200204000-00042.
24. Moore CL. Time to abandon the term "3 in 1" block. Annals of Emergency Medicine. 2015;66(2):215.

Original Article
Acta Medica Academica 2022;51(1):21-28
DOI: 10.5644/ama2006-124.366

Correlation of Surfactant Protein-D (SP-D) Serum Levels with ARDS Severity and Mortality in Covid-19 Patients in Indonesia

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Received: 1 November 2021; **Accepted:** 18 February 2022

Abstract

Objective. The purpose of this research was to investigate the correlation between serum levels of surfactant protein-D (SP-D) with acute respiratory distress syndrome (ARDS) severity and mortality in COVID-19. **Materials and Method.** This was a prospective cohort research study that included 76 patients in the period from July to October 2020. SP-D serum levels were taken upon admission to the hospital, the diagnosis of ARDS and its grade were confirmed according to the WHO criteria, and then patients were observed for 28-day mortality. **Results.** The mean SP-D serum levels from 76 patients were 39.33 ng/ml (SD±31.884 ng/ml). The statistical analysis showed that there was a significant correlation between SP-D serum levels and the severity of ARDS upon admission to the hospital (P=0.04, Spearman's rank correlation coefficient (rs)=0.26), but the correlation between serum levels of SP-D and mortality was not statistically significant (P=0.89; rs=-0.016). **Conclusion.** SP-D serum levels had a significant but weak correlation with ARDS severity, but were not significant for mortality.

Key Words: Surfactant Protein-D ▪ ARDS ▪ Severity ▪ Mortality ▪ COVID-19.

Introduction

In late 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), caused an epidemic of an acute respiratory disease in Wuhan, China. WHO later referred to this disease as Coronavirus Disease 2019 (COVID-19) (1), and as of September 2021 the number of people diagnosed with COVID-19 worldwide surpassed 221 million. Acute Respiratory Distress Syndrome (ARDS) is the most common complication of COVID-19 (60-70% of patients admitted to the ICU), followed by shock (30%), myocardial dysfunction (20-30%) and acute kidney injury (10-30%) (2). An initial study in China reported that ARDS in COVID-19 had a 28-day mortality rate of 74% (3).

The severity of the lung injury often requires mechanical ventilation, and recently some morphological pathways of COVID-19-related ARDS have been elucidated in a series of autopsies. Histological analysis of pulmonary vessels showed widespread thrombosis with microangiopathy, diffuse alveolar damage, capillary congestion, necrosis of pneumocytes, interstitial and intra-alveolar edema, and platelet–fibrin thrombi. These results suggest that the profound hypoxemia that these patients might experience can be due to both epithelial and endothelial injury (4). A study by Sega et al. showed that endothelial markers (especially Endoglin and VCAM-1) are associated with mortality in COVID-19 cases (5).

Surfactant Protein-D (SP-D) is a biomarker of lung epithelial injury, which is produced by type-II alveolar cells and plays an important role in maintaining the integrity of the alveolar-capillary interface. The mechanism of SP-D in the alveolar entering the circulation system is not fully understood, but Hart et al. suggested that in inflammatory conditions, such as ARDS, increased alveolar-capillary permeability may lead to leakage

of alveolar SP-D into the systemic circulation (6). SP-D is also thought to be a more specific marker for lung damage, so it can be used as a marker of early lung injury.

Several studies on SP-D have been conducted. One of them is a 2009 study by Delgado et al. that aimed to determine the correlation between SP-D and mortality in H1N1 virus infection. The results showed that higher SP-D serum levels were associated with the risk of death in patients with H1N1 pneumonia (7). Moreover, a recent study by Kerget et al. and Saito et al. examined SP-D in COVID-19 patients. These studies compared SP-D serum levels in a group of COVID-19 patients who had ARDS, and those who did not. SP-D, as a pulmonary epithelial maker, has been shown to be a prognostic biomarker that can predict the outcome of H1N1 virus infection and in non-COVID-19 cases, but there are not many studies about the role of SP-D in COVID-19.

The purpose of this study was to analyze the correlation between SP-D serum levels with ARDS severity and mortality in COVID-19 cases.

Method

This was a prospective cohort research study that included 76 patients. The study was performed in the COVID-19 isolation ward and ICU at Dr Soetomo Surabaya Hospital, in the period from July to October 2020, and was approved by the Ethics Comittee of Dr Soetomo Surabaya Hospital.

The study population consisted of patients treated in isolation wards and ICU at Dr Soetomo Surabaya Hospital in the period from July 2020 - October 2020. All patients who met the inclusion, and exclusion, criteria were included in the study sample until the minimum number of samples was met. The inclusion criteria were: confirmed diagnosis of COVID-19 and aged 18 years old or above. The exclusion criteria were: patients with COPD, asthma and tuberculosis infection; patients with an autoimmune disease, immunocompromised or those taking immunosuppressant drugs; and patients with malignancy or receiving chemotherapy treatment. The correlation sample size formula was used to calculate the minimum number of patients (α: 0.05; β: 0.2 and r: 0.5), and the minimum sample size was 30 patients.

The patients underwent a COVID-19 test, blood gas analysis and SP-D serum levels upon hospital admission. Diagnosis of COVID-19 was confirmed by an RT-PCR test from the patients' nasopharyngeal swab samples; the PaO_2/FiO_2 ratio was used to determine ARDS severity; and the ELISA method (Human SP-D ELISA Kit by Elabscience®) was used to determine SP-D serum levels. The definition of ARDS and its severity were categorized using the WHO criteria: a PaO2/FiO2 ratio of <100 as severe ARDS, 100-200 as moderate ARDS, 200-300 as mild ARDS and >300 was categorized as not ARDS (6). The diagnosis of ARDS was made upon hospital admission by history taking, physical examination and laboratory tests. All the patients received Dr Soetomo Surabaya Hospital standard protocol therapy for COVID-19 that follows the WHO's guidelines for Clinical Management of COVID-19.

Statistical Analysis

The IBM SPSS Statistics 23.0® program was used for statistical analysis. Continuous data were reported as means. Categorical data were presented as a percentage. The correlation between SP-D serum levels and ARDS severity and mortality were analyzed using the Spearman correlation test. A P value <0.05 was defined as statistically significant.

Results

Characteristics of Research Subjects

From 76 patients studied, 68 patients (89.5%) had comorbidities and 8 patients (10.5%) had no comorbidities. The most common comorbidities were obesity (64.7%), diabetes mellitus (48.5%) and hypertension (30.9%).The 51-60 years age group had the most patients who died (35.9%). However, when viewed on the basis of the proportion of the number of patients who survived and died in each age group, the >71 years age group was the worst, with a 100% mortality rate.

Table 1. Characteristics of Subjects

Variables	Survivor (N=39)	Non Survivor (N=37)	P value
Age (years) Mean±SD	55.56±12.96*	49.43±10.29*	0.026
Gender			
Male N (%)	25 (64.1)	21 (56.8)	0.513
Female N (%)	14 (35.9)	16 (43.2)	
BMI (kg/m^2)	26.67 (22.89-29,38)†	26.04 (22.72-28.01)†	0.323
SP-D (ng/mL)	24.48 (15.0-75.18)†	24.07 (13.40-63.58)†	0.775
PaO2/FiO2 ratio	117.8 (85.0-151.40)†	206.7 (121.75-300.35)†	<0.001
LOS (days)	7 (4-13)	22 (15-26.5)	<0.001

Data are reported as number (percentage); *Mean±standard deviation; Median [interquartile range] as appropriate. SD=Standard Deviation; BMI=Body Mass Index; SP-D=Surfactant Protein-D; PaO2=Partial Oxygen Pressure in artery; FiO2=Fraction of Inspired Oxygen; LOS=Length of Stays.

SP-D Serum Levels, ARDS Severity and Mortality

In this study, an analysis was also carried out to see the differences in the value of serum SP-D levels in patients who died and those who survived for up to 28 days of observation. The results obtained were the ARDS severity upon hospital admission (Table 2) and mortality after 28 days of observation (Table 3).

Table 2. Severity of ARDS

Severity	N (%)	PaO2/FiO2 ratio Mean±SD	SP-D (ng/mL) Mean±SD
Non ARDS	11 (14.5)	369.14±41.57	37.46±32.95
Mild ARDS	14 (18.4)	230.81±23.46	29.87±26.63
Moderate ARDS	29 (38.2)	145.03±30.10	32.67±30.35
Severe ARDS	22 (28.9)	81.30±12.29	55.04±32.62

PaO2=Partial Oxygen Pressure in artery; FiO2 Fraction of Inspired Oxygen; SD=Standard Deviation; SP-D=Surfactant Protein-D; ARDS=Acute Respiratory Distress Syndrome.

Table 3. Mortality of Research Subjects

Outcome	N (%)	SP-D (ng/mL) Mean ± SD
Survived	37 (48.7)	38.92±32.00
Died	39 (51.3)	39.70±32.18
Died <14 days	30 (39.5)	37.74±31.69
Died >14 days	9 (11.8)	46.22±34.87

SD=Standard Deviation; SP-D=Surfactant Protein-D.

Correlation between SP-D Serum Levels and ARDS Severity in COVID-19 Patients

Statistical analysis was performed to determine the significance of the correlation between SP-D serum levels and the severity of ARDS in this study. The results of the Spearman correlation analysis showed a correlation coefficient (rs) of 0.236 with a P value=0.04. This means that there was a significant correlation between SP-D serum levels and the severity of ARDS upon hospital admission (P<0.05), with a weak correlation strength (rs: 0.20 - 0.39).

Correlation between SP-D Serum Levels with Mortality in COVID-19 Patients

Statistical analysis was calculated to see the significance of SP-D serum levels with mortality in this study. The results of the Spearman correlation analysis showed a correlation coefficient (rs) of -0.016 with P value = 0.89. This means that the correlation between SP-D serum levels upon hospital admission and mortality was not significant (P>0.05), with a very weak negative correlation strength (rs: 0.00 to -0.19).

Discussion

This study highlights that SP-D had a connection with ARDS severity in COVID-19, thus supporting the opinion that treatment using surfactants

can provide benefits in COVID-19 cases. The purpose of our study was to give us a better understanding of the role of SP-D serum levels in COVID-19. We use 3 variables in this study: SP-D serum levels upon hospital admission; ARDS severity upon hospital admission; and mortality after 28 days of observation. The results of this study concluded that the correlation between SP-D serum levels upon hospital admission with ARDS severity upon hospital admission was statistically significant, with a weak correlation strength, but the correlation between SP-D serum levels with mortality was not statistically significant, with a very weak negative correlation strength.

Characteristics of the Research Subjects

The gender characteristics of this study was that it was dominated by male patients. The characteristics of these patients are similar to the study conducted by Zhi et al. where 63.8% of the 1023 confirmed COVID-19 patients studied were male. Male patients also dominated in the non-survivor group,which is similar to the systematic review conducted by Yustinawati et al. It was found that of a total of 1,314 COVID-19 patients who died, 845 patients (64%) were male (8). Gender is a risk factor for severe COVID-19. Sexual hormone-mediated immune responses and differences in ACE2 expression in the different sexes are thought to play a role in determining disease severity. It was explained that the females had a more effective adaptive immune response than the males. This may be due to the production of sex hormones and the difference in the number of genes related to immunity that are found more on the X chromosome (9, 10).

Age is a significant risk factor for COVID-19 because it is also associated with comorbidities and a decrease in the effectiveness of the immune system due to the physiological process of aging. A meta-analysis conducted by Booth et al. found that patients >75 years of age had a higher risk of contracting COVID-19 (11). A retrospective multicenter cohort study by Luo H et al., found that of 625 COVID-19 patients, there were 41.8% young adults (19 – 44 years old), 39.7% middle-aged adults (45 - 64 years old) and 12.6% elderly (≥ 65 years). In our study, the age of the patients was dominated by the 51 – 60 year age group and 41 – 50 years, which has similarities with the study by Luo et al (10). Elderly patients (≥ 65 years) with COVID-19 have the highest risk for severe or critical illness, intensive care, respiratory failure and length of hospital stay; which may be due to the higher incidence of comorbidities and decreased immunity to COVID-19 (12). Aging has been associated with modifications in signaling mechanisms responsible for IFN production, leading to reduced IFN production. Coronavirus, and specifically SARS-COV-2 infections, have been shown to induce IFN production poorly, a mechanism of possible viral immune escape. These immunological deficiencies can synergistically cooperate in a single patient leading to impaired IFN production, insufficient immune responses, and more severe manifestation of COVID-19 (13).

Obesity, diabetes mellitus and hypertension were the most common comorbidities suffered by the patients in this study. There is a clear correlation between obesity and basal inflammatory status, which is characterized by higher levels of IL-6 and CRP. Adipose tissue in obesity is "pro-inflammatory" and thus leads to increased expression of cytokines, especially adipokines. In the study by Roncon et al., it was reported that patients with diabetes mellitus had a nearly three times higher risk of admission to the ICU and death (14). A study by Chang et al. found from a multivariate logistic analysis that diabetes mellitus was significantly associated with disease progression of COVID-19. Diabetes is related to the progression of COVID-19 because hyperglycemia causes immune dysfunction, impaired neutrophil function, antioxidant system and humoral immunity, and causes a tendency to nosocomial infections that aggravate COVID-19 symptoms (15).Therapy for hypertension, diabetes mellitus and cardiovascular disease is needed to increase the expression of the ACE2 protein. In COVID-19 patients, SARS-CoV-2 binds to the ACE2 receptor, spreads and causes tissue damage, especially in organs with

high ACE2 receptor expression(16). This causes an increase in viral load which eventually aggravates the disease and then triggers ARDS, cytokine storm and even death (7).

SP-D Serum Levels in COVID-19 Patients

On the basis of the central role of SP-D in lung defense, regulation of the inflammatory response and its dysregulation in lung disease, it was hypothesized that increased levels of SP-D in blood serum are caused by lung tissue damage (7). The mechanism by which SP-D in the alveolar can enter the circulation system is not fully understood, but Hart et al. suggested that in inflammatory conditions, such as ARDS, increased alveolar-capillary permeability may lead to leakage of alveolar SP-D into the systemic circulation. The integrity of the secretory epithelial cells can be damaged in lung inflammation, resulting in leakage of SP-D from the epithelial cells into the alveoli and then into the blood vessels.

In this study, the overall mean SP-D serum level was lower than the mean value of SP-D in the H1N1 cases (39.33 ng/mL vs 434.5 ng/mL) that were studied by Delgado et al. This may be caused by the pathophysiological process of COVID-19 itself, whereas it is known that SARS CoV-2 enters the respiratory tract and binds to the ACE-2 receptor to enter type II alveolar cells. Type II alveolar cells' function is to produce surfactant, so the damage to these cells early in the course of the disease can decrease the amount of pulmonary surfactant. This may be the reason why the concentration of SP-D that leaks into the systemic circulation is not as high as with H1N1 infection.

Correlation between SP-D Serum Levels and ARDS Severity in COVID-19 Patients

A study conducted by Kerget et al. showed that in COVID-19 patients, day 0 SP-D levels were higher in patients with ARDS than without ARDS (P=0.001). However, the study by Kerget et al. only divided the patient groups into ARDS and non-ARDS, while the severity of ARDS was not elaborated. The study by Saito et al. showed that the SP-D serum levels showed a significant difference between the mild disease group (non-ARDS) and the severe disease group (ARDS) (P<0.001). SP-D serum level examinations were performed serially on days 3, 5 and 8. The increase in SP-D serum levels on day 8, when compared to day 3, was 8.5 times higher, and was also accompanied by the worsening of the clinical condition.

The results of our study showing that there was a significant correlation between SP-D serum levels and the severity of ARDS, but with weak correlation strength, are slightly different to the research by Kerget et al. and Saito et al. This may be due to differences in the characteristics of the study subjects (especially the type of race and comorbidities), and the day of SP-D serum collection. The study by Park et al. concluded that high SP-D serum levels within 48 hours of ICU admission serve as a diagnostic marker for ARDS and this is supported by the study by Saito et al., who found that the SP-D serum levels on day 8 had increased by about 8.5 times when compared to day 3 (17, 18). In our study, SP-D serum levels were measured on day 0, so that this could be the cause of the weak correlation strength.

In our study, 4 samples were found to be outliers. Of these 4 samples, a high SP-D value was obtained but the type of ARDS suffered was mild or moderate. This result contradicts the research by Kerget et al. and Saito et al. This may be due to the comorbidities suffered by these 4 patients who had allergies. The presence of an inflammatory process and injury to lung tissue affects the process of synthesis and secretion of SP-D from lung epithelial cells to the systemic circulation (19).

Koopmans et al. found an increase in SP-D levels in allergic patients compared to control subjects. SP-D levels increased 24 hours after allergen exposure in asthmatic patients, and were positively correlated with the number of eosinophils in the sputum. Thus, SP-D serum levels can serve as markers of the level of bronchial inflammation in allergic patients(20). Inhaled allergens are initially dissolved in the fluid lining the airways before they come into contact with immune cells. The airway

lining fluid contains surfactant, and therefore the initial contact between the allergen and the surfactant component will occur earlier. SP-A and SP-D interact with mite allergens via CRD, and inhibit the binding of allergen-specific IgE to mite allergens. This finding may indicate that SP-D inhibits the induction of allergic reactions by direct allergen binding, and thus is beneficial in reducing the binding of dendritic cells and allergen-specific IgE, thereby preventing acute asthma attacks(21).

Correlation between SP-D Serum Levels with Mortality in COVID-19 Patients

The analysis results of this study showed that there was no significant correlation between SP-D serum levels on day 0 with mortality. This result is different from the study by Kerget et al., where the SP-D serum levels in the group that died (7 patients) compared to those who survived (81 patients) were 96.7±37.2 ng/ml and 56.9±43.5 ng/ml (P=0.03) respectively (22).

The report by Ruan et al. on 68 COVID-19 patients who died in Wuhan showed that 53% died due to respiratory failure, 7% due to shock (possibly due to fulminant myocarditis), 33% due to both, and 7% from unclear mechanisms (23). Elezkurtaj et al. conducted an autopsy study on 26 COVID-19 patients who died in a Berlin hospital, with the aim of finding the exact causes of the deaths of these patients. The autopsy results found that the most common immediate causes of death were septic shock and/or multi-organ failure (30.8%), viral pneumonia with or without signs of bacterial superinfection (19.2%), respiratory failure due to diffuse alveolar damage or ARDS (19.2%), right ventricular heart failure (15.4%), and massive pulmonary thromboembolism, severe bronchial aspiration, gastrointestinal bleeding, or left ventricular heart failure (3.8% each). These two studies show that the most common causes of death in COVID-19 patients are ARDS and septic shock accompanied by multi-organ failure (24).

SP-D serum levels, as a pulmonary epithelial marker, have been shown to correlate with ARDS mortality in COVID-19 patients (25). Early identification of pulmonary epithelial injury is one way of detecting the early stages of Acute Lung Injury before it deteriorates to ARDS (19). SP-D serum levels are thought to be a more specific marker for

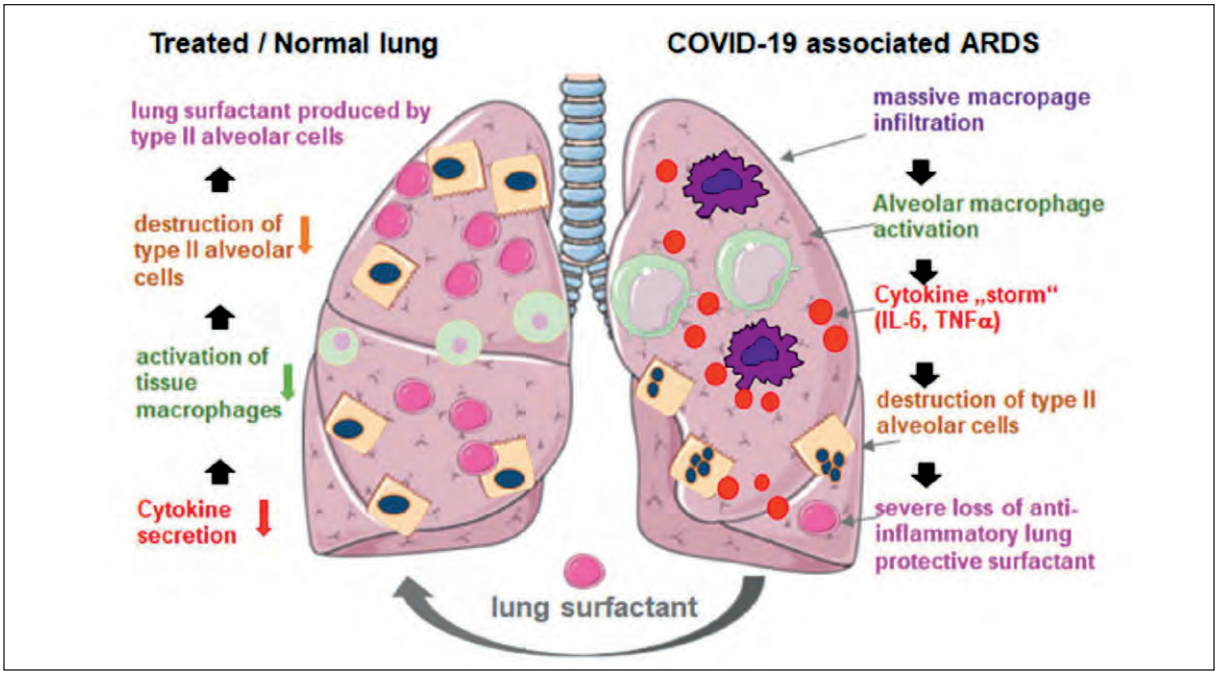

Figure 1. Hypothesis of Surfactant Therapy Effect Mechanism.

the damage that occurs in the lungs, but they are not specific for the damage that occurs in other organs.

In addition to the role of SP-D as a biomarker capable of describing the severity of ARDS in COVID-19, some opinions support the possibility that surfactant therapy may provide benefits in COVID-19 cases. Although pulmonary surfactant therapy is a standard, safe and effective therapy for neonates with ARDS, treatment with recombinant SP-C-based surfactant has not shown an increase in survival in a randomized controlled trial in adults (26). The use of natural surfactants seems to be more advantageous than synthetic surfactants in increasing blood oxygenation significantly and shortening ventilatory time in infant patients. Meconium aspiration syndrome resembles COVID-19 pneumonia in which there is decreased surfactant production due to the destruction of type II alveolar cells. Early administration of natural surfactants reduces the need for ECMO therapy and ventilatory time in infant patients. This suggests that initial administration of natural surfactants should also improve lung function in adult patients with severe ARDS. Thus, surfactant therapy in ARDS patients due to COVID-19 may be of benefit, especially when applied early in the disease course (Figure 1).

There are not many studies about the correlation between SP-D serum levels with ARDS severity and mortality in COVID-19 cases, so further research still needs to be done. The limitations of this study were the relatively low number of patients and the lack of adjustment for confounders. Using a control group and SP-D serum levels on collected serial days may be able to provide a better picture of the correlation between SP-D serum levels and the ARDS severity in COVID-19 patients.

Conclusion

From this study, it can be concluded that SP-D serum levels had a significant and weak correlation strength with ARDS severity, but not a significant correlation with mortality.

What Is Already Known on This Topic:

In our study, it was found that the mean serum level of SP-D in the survivors was 38.93 ng/mL, whereas in the deceased group it was 39.7 ng/mL. This indicates that there is no significant relationship between serum SP-D levels on day 0 with mortality (P= 0.89). This result is different from a previous study which stated that the serum SP-D levels in the dead group (7 patients) compared to the survivor group (81 patients) were 96.7 ± 37.2 ng/ml and 56.9 ± 43.5 ng. / ml (P= 0.03). Then, serum SP-D levels were considered as a more specific marker for lung damage, but not specific for other organ damage.

What This Study Adds:

The study used an analytic observational perspective with a prospective cohort study design, in which the study population were COVID-19 infection patients suffering from COPD, Asthma and TB infection; patients with autoimmune disease, immunocompromised or taking immunosuppressant drugs; and patients with malignancy or receiving chemotherapy treatment.

Authors' Contributions: Conception: AA and ASV; Design: AA, ASV, and AU; Acquisition, analysis and interpretation of data: AA, ASV, and AU; Drafting the article: AA; Revising it critically for important intellectual content: AA, ASV, and AU; Approved final version of the manuscript: AA, ASV, and AU.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, et al. Surviving Sepsis Campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). Intensive Care Med. 2020;46(5):854-87. doi: 10.1007/s00134-020-06022-5.
2. Phua J, Weng L, Ling L, Egi M, Lim CM, Divatia JV, et al. Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. Lancet Respir Med. 2020;8(5):506-17. doi: 10.1016/S2213-2600(20)30161-2.
3. Goh KJ, Choong MC, Cheong EH, Kalimuddin S, Duu Wen S, Phua GC, et al. Rapid Progression to Acute Respiratory Distress Syndrome: Review of Current Understanding of Critical Illness from Coronavirus Disease 2019 (COVID-19) Infection. Ann Acad Med Singap. 2020;49(3):108-18.
4. Spadaro S, Fogagnolo A, Campo G, Zucchetti O, Verri M, Ottaviani I, et al. Markers of endothelial and epithelial pulmonary injury in mechanically ventilated COVID-19 ICU patients. Crit Care. 2021;25(1):74. doi: 10.1186/s13054-021-03499-4.
5. Vieceli Dalla Sega F, Fortini F, Spadaro S, Ronzoni L, Zucchetti O, Manfrini M, et al. Time course of endothelial dysfunction markers and mortality in COVID-19 pa-

tients: A pilot study. Clin Transl Med. 2021;11(3):e283. doi: 10.1002/ctm2.283.

6. Hartl D, Griese M. Surfactant protein D in human lung diseases. Eur J Clin Invest. 2006;36(6):423-35. doi: 10.1111/j.1365-2362.2006.01648.x.
7. Delgado C, Krötzsch E, Jiménez-Alvarez LA, Ramírez-Martínez G, Márquez-García JE, Cruz-Lagunas A, et al. Serum Surfactant Protein D (SP-D) is a Prognostic Marker of Poor Outcome in Patients with A/H1N1 Virus Infection. Lung. 2015;193(1):25-30. doi: 10.1007/s00408-014-9669-3.
8. Yustinawati R, Achadi A. Risk Factors for Mortality in Patients with COVID-19: A Systematic Review. The 7th International Conference on Public Health, Solo, Indonesia, November 18-19, 2020 | 71. doi: https://doi.org/10.26911/the7thicph.01.26.
9. Ortona E, Pierdominici M, Rider V. Editorial: Sex Hormones and Gender Differences in Immune Responses. Front Immunol. 2019;10:1076. doi: 10.3389/fimmu.2019.01076.
10. Bastolla U. Mathematical Model of SARS-Cov-2 Propagation Versus ACE2 Fits COVID-19 Lethality Across Age and Sex and Predicts That of SARS. Front Mol Biosci. 2021;8:706122. doi: 10.3389/fmolb.2021.706122.
11. Booth A, Reed AB, Ponzo S, Yassaee A, Aral M, Plans D, et al. Population risk factors for severe disease and mortality in COVID-19: A global systematic review and meta-analysis. PLoS One. 2021;16(3):e0247461. doi: 10.1371/journal.pone.0247461.
12. Luo H, Liu S, Wang Y, Phillips-Howard PA, Ju S, Yang Y, et al. Age differences in clinical features and outcomes in patients with COVID-19, Jiangsu, China: a retrospective, multicentre cohort study. BMJ Open. 2020;10(10):e039887. doi: 10.1136/bmjopen-2020-039887.
13. Contoli M, Papi A, Tomassetti L, Rizzo P, Vieceli Dalla Sega F, Fortini F, et al. Blood Interferon-α Levels and Severity, Outcomes, and Inflammatory Profiles in Hospitalized COVID-19 Patients. Front Immunol. 2021;12:648004. doi: 10.3389/fimmu.2021.648004.
14. Roncon L, Zuin M, Rigatelli G, Zuliani G. Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome. J Clin Virol. 2020;127:104354. doi: 10.1016/j.jcv.2020.104354.
15. Ho W-P, Liau JJ, Cheng CK. Biomechanical study of bone-patellar tendon-bone and bone-ACL-bone grafts. J Med Biol Eng. 2002;22(2):103-8.
16. Zhang H, Zhang Y, Wu J, Li Y, Zhou X, Li X, et al. Risks and features of secondary infections in severe and critical ill COVID-19 patients. Emerg Microbes Infect. 2020;9(1):1958-64. doi: 10.1080/22221751.2020.1812437.
17. Chang MC, Park YK, Kim BO, Park D. Risk factors for disease progression in COVID-19 patients. BMC Infect Dis. 2020;20(1):445. doi: 10.1186/s12879-020-05144-x.
18. Saito A, Kuronuma K, Moniwa K, Kodama K, Takahashi S, Takahashi H, et al. Serum surfactant protein A and D may be novel biomarkers of COVID-19 pneumonia severity. Research Square. 2020:May:1-17. [preprint]
19. Veterini AS. A primary biomarker examination in preventing progressivity of acute respiratory distress syndrome: The role of surfactant protein-d in sepsis induced ARDS. Crit Care Shock. 2020;23(2):65-75.
20. Koopmans JG, van der Zee JS, Krop EJ, Lopuhaä CE, Jansen HM, Batenburg JJ. Serum surfactant protein D is elevated in allergic patients. Clin Exp Allergy. 2004;34(12):1827-33. doi: 10.1111/j.1365-2222.2004.02083.x.
21. Hohlfeld JM, Erpenbeck VJ, Krug N. Surfactant proteins SP-A and SP-D as modulators of the allergic inflammation in asthma. Pathobiology. 2002-2003;70(5):287-92. doi: 10.1159/000070744.
22. Kerget B, Kerget F, Koçak AO, Kızıltunç A, Araz Ö, Uçar EY, et al. Are Serum Interleukin 6 and Surfactant Protein D Levels Associated with the Clinical Course of COVID-19? Lung. 2020;198(5):777-84. doi: 10.1007/s00408-020-00393-8.
23. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020;46(5):846-8. doi: 10.1007/s00134-020-05991-x.
24. Elezkurtaj S, Greuel S, Ihlow J, Michaelis EG, Bischoff P, Kunze CA,et al. Causes of death and comorbidities in hospitalized patients with COVID-19. Sci Rep. 2021;11(1):4263. doi: 10.1038/s41598-021-82862-5.
25. Spadaro S, Park M, Turrini C, Tunstall T, Thwaites R, Mauri T, et al. Biomarkers for Acute Respiratory Distress syndrome and prospects for personalised medicine. J Inflamm. 2019;16(1). doi: https://doi.org/10.1186/s12950-018-0202-y.
26. Meng SS, Chang W, Lu ZH, Xie JF, Qiu HB, Yang Y, et al. Effect of surfactant administration on outcomes of adult patients in acute respiratory distress syndrome: a meta-analysis of randomized controlled trials. BMC Pulm Med. 2019;19(1):9. doi: 10.1186/s12890-018-0761-y.

Original Article
Acta Medica Academica 2022;51(1):29-34
DOI: 10.5644/ama2006-124.367

Clopidogrel Resistance Among Ischemic Stroke Patients and Its Risk Factors in Indonesia

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Received: 5 January 2022; **Accepted:** 25 April 2022

Abstract

Objective. Clopidogrel is a common antiplatelet used as secondary prevention of ischemic stroke, known to have better efficacy than aspirin, with a equivalent safety profile. However, clopidogrel resistance is not uncommon but has not been widely studied in Asia. This study will further assess clopidogrel resistance and its risk factors. **Materials and Methods.** A cross-sectional study was conducted at Rumah Sakit Universitas, Indonesia, and Rumah Sakit Cipto Mangunkusumo, Indonesia in 2020-2021. All patients had had at least one episode of ischemic stroke. Clopidogrel resistance was assessed using a VerifyNow assay. **Results.** 57 subjects were enrolled in this study. We found 15.8% of subjects were clopidogrel resistant. Gender was significantly associated with clopidogrel resistance, with males having 80% lower clopidogrel resistance (OR 0.2 (95% CI 0.022 – 0.638); P=0.006). Meanwhile, smoking was not associated with clopidogrel responsiveness (P=0.051). We found no association between haemoglobin, blood glucose, HbA1c, cholesterol, liver enzymes, serum urea concentration or creatinine levels and clopidogrel resistance. **Conclusion.** Clopidogrel remains an effective treatment to prevent recurrent ischemic stroke in Indonesia. Further studies are needed to assess gene polymorphism and clopidogrel resistance, which may explain the findings of this study.

Key Words: Clopidogrel Resistance ▪ Ischemic Stroke ▪ Risk Factors.

Introduction

The American Heart Association/American Stroke Association (AHA/ASA) guidelines for the secondary prevention of ischemic stroke recommend the administration of antiplatelet therapy over anticoagulation, to reduce the recurrence of non-cardioembolic ischemic stroke. Among the recommended antiplatelet therapies are aspirin 50 to 325 mg daily, clopidogrel 75 mg, or a combination of aspirin 25 mg and extended-release dipyridamole 200 mg, twice daily (1). It is also said that in patients with a recent minor stroke (NIHSS ≤3) and high-risk of transient ischemic attack (TIA; ABCD2 score ≥4), the combination of aspirin and clopidogrel should be initiated early, within 90 days, followed by single antiplatelet therapy for the prevention of ischemic stroke (2). However, not all individuals respond to aspirin, leading to recurrent cardiovascular events, despite ongoing therapy. Aspirin resistance was shown in 22.5-26.4% of individuals with cardiovascular disease, and even more in Asia, with 27.3% showing aspirin resistance (3).

Clopidogrel is a widely used antiplatelet for secondary prevention of stroke and coronary heart disease. It works by irreversibly modifying the adenosine diphosphate (ADP) receptor (P2Y12 receptor) on platelets, thus inhibiting platelet activation (4). It is also commonly used in Indonesia since it is a widely available alternative to aspirin. The CAPRIE study showed that clopidogrel is superior

in efficacy to aspirin, with a relatively equivalent safety profile (5). While aspirin resistance is common and widely known, there have not been many studies assessing clopidogrel resistance, especially in Indonesia. This study aims to assess the prevalence of clopidogrel resistance among ischemic stroke patients in Indonesia, and its risk factors.

We hope that this study will present the characteristics of patients who have a higher risk of clopidogrel resistance, thus providing awareness and alternative treatments for such populations.

Methods

This is a cross-sectional study performed at Rumah Sakit Universitas, Indonesia, and Rumah Sakit Cipto Mangunkusumo in Indonesia. All stroke patients in 2020-2021 who fulfilled the criteria for this research, which included ischemic stroke, whether primary or recurrent, age between 40 to 80 years, no history of kidney disease, bleeding disease or atrial fibrillation, and who had never taken anticoagulants before, and did not consume omeprazole, esomeprazole, or atorvastatin. The patients were given clopidogrel for five days, and a blood sample was taken to be examined for VerifyNow levels, which indicated clopidogrel resistance by the P2Y12 reaction unit (PRU) value. VerifyNow results were categorized using two different methods: the first method classified clopidogrel resistance as unresponsive (PRU >235) and responsive (PRU ≤235), while the second method classified clopidogrel resistance as unresponsive (PRU >208), responsive (PRU ≤208) and a bleeding risk (PRU <95). Clopidogrel resistance or responsiveness was considered as high on-treatment platelet reactivity (HTPR). HTPR may reflect the pharmacodynamics of clopidogrel on platelets because the cause of HTPR is the same as the cause of low response and resistance.

Ethics Statement

This research was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital, approval number KET-658/UN2/F1/ETIK/PPM.00.02/2020.

Statistical Analysis

Statistical analysis was conducted using IBM SPSS version 25. Statistical tests were performed using Chi-square, one-way ANOVA, student's T-test and Kruskal-wallis. A significant result was defined as $P<0.05$. Logistic regression analysis was used to assess the associations with responsiveness to clopidogrel.

Results

In this study, we analysed 57 subjects who had taken Clopidogrel for at least 5 days for ischemic stroke prevention. Using the first method (unresponsive with PRU >235), we found 9 subjects (15.8%) who met the HTPR criteria (clopidogrel resistance) and 48 subjects (84.2%) who responded to clopidogrel. Bivariate analysis showed that several risk factors are associated with clopidogrel resistance, such as female gender and height (Table 1).

Moreover, we found that females generally had higher PRU levels than males, while smoking history, diabetes mellitus, hypertension, dyslipidemia, stroke history, and fatty liver were not significantly associated with VerifyNow PRU levels (Table 2).

We further analysed several serum markers that may be associated with clopidogrel resistance using the second method classifying VerifyNow results as unresponsive (PRU >208), responsive (PRU ≤208), and bleeding risk (PRU <95). No association was found between laboratory results and clopidogrel resistance or bleeding risk.

A significant association between gender and clopidogrel resistance was identified, and it was also shown that female had a higher chance to be resistant to clopidogrel than men ($P=0.006$). Further, the data from VerifyNow showed that stroke patients who smoke had lower VerifyNow levels compared to people who do not smoke, which suggested they were still responsive to clopidogrel although not reaching the bleeding risk level.

Table 1. Baseline Characteristics

Characteristics	Unresponsive N=9; (%)	Responsive N=48; (%)	P-value	Mean difference/OR (95% CI)
Gender				
Male	2 (22.2)	34 (70.8)	0.006*	0.2 (0.022 - 0.638)
Female	7 (77.8)	14 (29.2)		
Smoking				
Yes	1 (11.1)	22 (45.8)	0.051*	6.76 (0.785 - 58.404)
No	8 (88.9)	26 (54.2)		
DM				
Yes	3 (33.3)	15 (31.3)	0.902*	0.909 (0.200 - 4.133)
No	6 (66.7)	33 (68.8)		
Hypertension				
Yes	7 (77.8)	37 (77.1)	0.964*	0.961 (0.174 - 5.311)
No	2 (22.2)	11 (22.9)		
Dyslipidemia				
Yes	7 (77.8)	30 (62.5)	0.378*	0.476 (0.089 - 2.546)
No	2 (22.2)	18 (37.5)		
Dyspepsia				
Yes	2 (22.2)	10 (20.8)	0.925*	0.921 (0.165 -5.138)
No	7 (77.8)	38 (79.2)		
History of stroke				
Yes	3 (33.3)	18 (37.5)	0.812*	1.2 (0.267 – 5.400)
No	6 (66.7)	30 (62.5)		
Fatty liver				
Yes	5 (55.6)	21 (43.8)	0.514*	0.622 (0.148 - 2.608)
No	4 (44.4)	27 (56.3)		
Age	53.89 (±9.82)	55.56 (±11.10)	0.675†	1.673 (9.631 - 6.284)
Blood pressure				
Systolic	141.88 (±20.18)	137.29 (±31.44)	0.675†	4.59 (-12.58 - 21.77)
Diastolic	78.55 (±13.70)	79.79 (±11.05)	0.768†	1.23 (-12.01 - 9.53)
Weight	59 (±11.78)	67 (±14.96)	0.137†	8 (18.64 - 2.64)
Height	156.67 (±6.55)	162.11 (±7.18)	0.040†	2.58 (10.63 - 0.25)

*Chi-square; †One-way ANOVA.

Table 2. VerifyNow Levels by Groups

Characteristics	VerifyNow	P-value*
Gender		
Male	120.72 (±63.35)	0.036
Female	164.76 (±79.06)	
Smoking		
Yes	116.34 (±61.82)	0.076
No	150.88 (±76.03)	
DM		
Yes	146.44 (±73.62)	0.504
No	132.56 (±71.94)	
Hypertension		
Yes	136.06 (±74.68)	0.867
No	139.92 (±74.68)	
Dyslipidemia		
Yes	141.97 (±78.88)	
No	127.65 (±58.3)	0.479
Stroke History		
Yes	141 (±65.03)	0.749
No	134 (±76.73)	
Fatty liver		
Yes	142.65 (±78.36)	0.589
No	132.16 (±67.36)	

DM=Diabetes mellitus type 2; *Independent T-test.

Table 3. Laboratory Findings and Clopidogrel Resistance

Laboratory findings	Unresponsive N=12	Responsive N =31	Bleeding risk N =14	P-value
Haemoglobin (g/dL)	12. 48 (±1.85)	13.8 (±2.83)	14.02 (±2.27)	0.269*
FBG (mg/dL)	94.5 (80 – 280)	113 (73.7 - 331)	94.6 (77 - 449)	0.389†
HbA1c (%)	5.6 (4.8 – 11.8)	5.7 (4 - 12.3)	5.35 (4.4 - 15)	0.715†
Total Cholesterol (mg/dL)	193 (±47.4)	183.77 (±55.24)	196.42 (±54.26)	0.730*
HDL Cholesterol (mg/dL)	56.08 (±23.7)	48.67 (±10.81)	47.92 (±14.66)	0.307*
LDL Cholesterol (mg/dL)	133 (±50.42)	125.35 (±45.15)	126.07 (±45.47)	0.882*
Triglyceride (mg/dL)	147.5 (61 - 503)	150 (57 – 497)	140.5 (57-199)	0.596†
AST (IU/L)	18.67 (±7.64)	19.5 (±6.86)	19.07 (±5.83)	0.933*
ALT (IU/L)	20 (6 – 35)	21 (9 - 50)	19.5 (8 - 58)	0.756†
Urea (mg/dL)	30 (16 – 54)	27 (7 -168.4)	27 (17 - 55)	0.657†
Creatinine (mg/dL)	0.9 (0.5 – 3)	1 (0.48 - 17.8)	0.85 (0.5 - 2.48)	0.679†

FBG=Fasting Blood Glucose; HbA1c=Haemoglobin A1c; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; *One Way ANOVA; †Kruskal-Wallis.

Discussion

This study found that 15.8% ischemic stroke patients were resistant to clopidogrel. A significant association was found between gender and clopidogrel resistance (p=0.006) showing that males have 80% lower chance of having clopidogrel resistance. These results were slightly different than previous studies, which found that clopidogrel resistance in stroke patients varied from 28% to 44%. Some possible mechanisms have been suggested for antiplatelet resistance. These are generally divided into two mechanisms, the first being due to inadequate inhibition of COX-1 or P2Y12, and the second antiplatelet resistance despite adequate inhibition of COX-1 or P2Y12 (6, 7).

The first mechanism may occur due to a decrease in bioavailability caused by poor compliance, inappropriate dosing, a decrease in absorption, drug interaction, and the high metabolism of clopidogrel. Aside from bioavailability, gene polymorphism related to the P2Y12 receptor gene and CYP3A4, CYP1A2, CYP2C19 may also cause clopidogrel resistance. As for the second mechanism, it may be due to the activation of bizarre platelet stimulation pathways, including an increase in epinephrine mediated platelet activation, COX-2 expression induced by stress in platelets, high ADP and collagen platelet sensitivity, high release of ADP, and excessive activation of platelets induced by red cells. Another possible aetiology includes high platelet turnover induced by stress, surgery, acute ischemic or inflammation.

Currently there are no exact standards or techniques for measuring antiplatelet resistance. Some platelet function examinations are used, such as VerifyNow, Platelet Function Analyser and Thromboelastography, which are used to assess clopidogrel resistance in clinical studies. However, there are limitations to the interpretation of the results. Meanwhile, susceptibility to clopidogrel resistance is extremely important, as a study by Xi et al. showed that stroke patients with clopidogrel resistance may exhibit early neurological degradation and more frequent recurrence of ischemic stroke, with poor recovery of neuron cells (3, 4).

The associations between several risk factors that cause clopidogrel resistance were studied by Patel et al., including age, gender, concomitant drugs, duration of antiplatelet therapy, and NSAID and statin consumption (8). On the basis of their study, it is known that there is no significant association between clopidogrel resistance and those factors. Our study also showed that there is no significant association between age and clopidogrel resistance. However, in contrast to Patel et al., our study found that there was a significant association between gender and clopidogrel resistance (P=0.006). Furthermore, we found that men

have an 80% lower chance of having clopidogrel resistance (OR 0.2; 95% CI 0.022 – 0.638). This finding is also supported by the fact that women tend to show higher VerifyNow levels than men (P=0.036). A plausible reason for these findings is that there is a relationship between clopidogrel resistance and the gene expressed in the sex chromosome. Therefore, further studies in gene polymorphism are needed to confirm these findings.

Kang et al. found that there was no significant relationship between smoking and clopidogrel resistance (9). That finding is in contrast to another study by Maruyama et al. that showed that ischemic stroke patients who were smokers had a lower chance of developing clopidogrel resistance compared to patients who were not smokers (10). To emphasize, this means that the responsiveness of clopidogrel is enhanced in patients who are smokers. Maruyama et al. also explained in their previous finding that smokers have a higher level of VerifyNow scores than people who do not smoke (10).

Our study had the same perspective as Kang et al.'s study that smoking was not significantly associated with the VerifyNow levels (9). Additionally, we observed that, contrary to Maruyama et al.'s study, smokers had a lower VerifyNow score compared to non-smokers (P=0.076) (10). The explanation of these findings is still inconclusive since there are not many studies that have explained this association. There were other factors that this study observed, such as diabetes mellitus, hypertension, and atrial fibrillation, which were found to be insignificant (10). Kang et al. showed that diabetes mellitus is an independent risk factor for clopidogrel resistance (9). However, in our study, we found no association between HbA1c or fasting blood glucose levels and clopidogrel resistance.

Limitations of the Study

The main limitation of this study is the relatively small number of patients.

Conclusion

In conclusion, our study showed that 15.8% of patients in the population studied suffered from clopidogrel resistance, where women had a higher risk of clopidogrel resistance. We also found no association between smoking and clopidogrel resistance (P=0.051). Overall, we can say that clopidogrel is still potent for ischemic stroke patients since our data found that the majority of patients (84.2%) showed responsiveness to clopidogrel, based on VerifyNow results.

What Is Already Known on This Topic:
The superiority of clopidogrel as a treatment for some diseases such as stroke and coronary heart disease since it demonstrates better safety than previous treatments, such as aspirin.

What This Study Adds:
Clopidogrel resistance and its association with some risk factors.

Acknowledgement: The authors would like to thank Universitas Indonesia for funding this research through the PUTI Grant. contract number NKB-2282/UN2.RST/HKP.05.00/2020

Authors' Contributions: Conception and design: RH, RAN, AR, SH, ARH, H, ML, EL, ASR, TL; Acquisition, analysis and interpretation of data: RH, RAN, SH, H; Drafting the article: RH, RAN, AR; Revising it critically for important intellectual content: ARH, H, ML, EL, ASR, TL; Approved final version of the manuscript: SH, ASR, TL

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Kleindorfer DO, Towfighi A, Chaturvedi S, Cockroft KM, Gutierrez J, Lombardi-Hill D, et al. 2021 Guideline for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack: A Guideline From the American Heart Association/American Stroke Association. Stroke. 2021;52(7):e364-467. doi: 10.1161/STR.0000000000000375.
2. Wang Y, Wang Y, Zhao X, Liu L, Wang D, Wang C, et al. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. N Engl J Med. 2013;369(1):11-9. doi: 10.1056/NEJMoa1215340.
3. Ebrahimi P, Farhadi Z, Behzadifar M, Shabaninejad H, Abolghasem Gorji H, Taheri Mirghaed M, et al. Preva-

lence rate of laboratory defined aspirin resistance in cardiovascular disease patients: A systematic review and meta-analysis. Caspian J Intern Med. 2020;11(2):124-34. doi: 10.22088/cjim.11.2.124.

4. Wallentin L. P2Y(12) inhibitors: differences in properties and mechanisms of action and potential consequences for clinical use. Eur Heart J. 2009;30(16):1964-77. doi: 10.1093/eurheartj/ehp296.
5. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. Lancet. 1996;348(9038):1329-39. doi: 10.1016/s0140-6736(96)09457-3.
6. Fukuoka T, Furuya D, Takeda H, Dembo T, Nagoya H, Kato Y, et al. Evaluation of clopidogrel resistance in ischemic stroke patients. Intern Med. 2011;50(1):31-5. doi: 10.2169/internalmedicine.50.3713.
7. Fong J, Cheng-Ching E, Hussain MS, Katzan I, Gupta R. Predictors of biochemical aspirin and clopidogrel resistance in patients with ischemic stroke. J Stroke Cerebrovasc Dis. 2011;20(3):227-30. doi: 10.1016/j.jstrokecerebrovasdis.2009.12.004.
8. Patel S, Arya V, Saraf A, Bhargava M, Agrawal CS. Aspirin and Clopidogrel Resistance in Indian Patients with Ischemic Stroke and its Associations with Gene Polymorphisms: A Pilot Study. Ann Indian Acad Neurol. 2019;22(2):147-152. doi: 10.4103/aian.AIAN_4_18.
9. Kang HG, Shin YY, Heo SH, Chang D-I, Kim BJ. Smoking and Clopidogrel resistance in ischemic stroke (P1.3-007). Neurology. 2019;92(15 Suppl):P1.3-007.
10. Maruyama H, Fukuoka T, Deguchi I, Ohe Y, Horiuchi Y, Kato Y, et al. Relationship between Smoking and Responsiveness to Clopidogrel in Non-cardiogenic Ischemic Stroke Patients. Intern Med. 2014;53(22):2575-9.
11. Topçuoglu MA, Arsava EM, Ay H. Antiplatelet resistance in stroke. Expert Rev Neurother. 2011;11(2):251-63. doi: 10.1586/ern.10.203.

Original Article
Acta Medica Academica 2022;51(1):35-45
DOI: 10.5644/ama2006-124.368

Changes in Attitudes towards Organ Donation among Bosnian Immigrants in Sweden from Gender Perspective

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Received: 10 March 2022; **Accepted:** 26 April 2022

Abstract

Objectives. The present study aimed to assess changes in the perception of and willingness to participate in organ donation (OD) among immigrants from Bosnia and Herzegovina living in Sweden from the perspective of gender differences. **Materials and Methods.** A cross-sectional study with 60 participants born in Bosnia and Herzegovina and living in Sweden was performed. Data were collected using a self-administrative questionnaire, providing demographic characteristics, information about opinions, awareness, and knowledge on the donation process and religious approach to the subject, willingness to donate/receive organs, and possession of a donor card. **Results.** Our results showed significant differences between genders regarding the definition of transplantation (P<0.0001), information about OD (P<0.0001), knowledge (P<0.0001) and importance of OD (P<0.003), religious permitting (P=0.0001), and religious opposing (P=0.0007) to OD. Furthermore, a significant difference was observed regarding the preferred recipient (P=0.0062) and the possession of the donor card (P<0.0001). Regression analysis showed that female gender and higher income were statistically significant in prediction of positive attitudes toward OD (P=0.0027, P=0.0002, respectively). **Conclusion.** Change of social background and integration into Swedish society undoubtedly led to change in the attitudes toward OD, regardless of the perspective of gender differences. However, women were found to have more positive attitudes toward OD.

Key Words: Organ Donation ▪ Gender Difference ▪ Education Level, Knowledge ▪ Transplantation.

Introduction

Organ transplantation (OT) together with organ donation (OD) represents one of the most successful advances in modern medicine, that gives patients the opportunity for a new life and survival through these processes (1). Organ donation (OD) is a personal choice and many ethical, legal, medical, organizational, and social factors are involved (2, 3).

In general, organs can be utilized from either living or deceased donors. The shortage of donated organs is a globally increasing problem. It becomes clear that the only way to overcome this issue is to increase the number of deceased donors (4). In 2018 organ donations globally reached the number of 146 840 (5). In 2019, before the global pandemic of SARS-COV-2, Spain had the highest number of deceased donors per million population (pmp) in Europe (49.6 pmp), followed by Croatia (36.4 pmp). At the same time, Turkey had the highest number of living donors (53.2 pmp). Meanwhile, Sweden had 19.2 deceased and 14.5 living donors pmp, far less than leading countries. Along with Kosovo, one of the lowest OD rates in Europe in 2017 had Bosnia and Herzegovina with 0.9 deceased and 5.9 living donors pmp (6).

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Many reasons and factors affect a person's decision to be an organ donor. Religion plays an important role, as well as race, gender and age (7-12). In most cases, better education and higher salaries lead to more organ donations (11, 13). Immunological factors and other non-immunological issues, including the organ size (14), donor's age (15), and weight (16), may also be involved. Other more specific factors as gender may also affect a person's stance and decision to donate an organ (17). Studies showed that women are more willing to donate but less willing to receive transplantations (18, 19). There are gender differences regarding OD in the context of heart (20), lung (21), kidney (22), and bone marrow transplants (23).

It is presumed that gender differences are a multifactorial issue. There is a greater need for transplants due to end-stage disease in men as they are more prone to hypertension and ischemic heart disease (20). This also means that they more often represent borderline or ineligible candidates for OD (20-22). However, women appear to know less about transplantation. This may be because they rarely undergo aggressive medical treatment but it should also be taken into account the different roles that women play in society, the economy, and culture (24). These differences are pronounced through the importance of achieving a suitable social climate for donation. Subsequently, a change in the social environment can positively influence the attitudes and perception of available information.

The present study aimed to assess changes in the perception of and willingness to participate in OD among immigrants from Bosnia and Herzegovina living in Sweden from the perspective of gender differences.

Methods

Study Design

The study was conceived as a quantitative cross-sectional study with a descriptive design using data from a self-administered questionnaire.

Participants

A study was performed among the participants from Bosnia and Herzegovina living in two cities in the western part of Sweden. The inclusion criterion was participants who were older than 18 years and willing to participate. We excluded individuals with cognitive impairment and individuals who required OT. We asked 72 people to participate in the present study, of which 12 declined due to lack of time and unwillingness. As a result, our final sample included 60 participants, 30 men and 30 women. The questionnaires and all communication were carried out in Bosnian. All the participants provided signed informed consent before completing the questionnaires. The participation was voluntary, and respondents could withdraw their consent at any time without incurring penalties or any loss of access to services. The demographic and clinical characteristics of the informants are shown in Table 1.

Data Collection

The questionnaire was specifically designed and administrated by the authors to achieve the aim of this study. The questions were organized into four sections. The first section contained sociodemographic details of the participants, such as age, gender, educational level, religion, income and marital status. The second section focused on the participants' awareness regarding OD, legislation, their opinions, promotion of OD and sources of information on the subject. The third section contained the question about participants' medical knowledge, knowledge about the donation process, possession of donor card, together with knowledge about a religious approach to OD. The last section of the questionnaire aimed to attain information about participants' willingness to donate their organs. In the last part, the participants were asked to write whether they would donate organs to family members, relatives, neighbors, or to anybody. Possession of a donor card was taken as

the main indicator of a positive attitude towards OD. Validation was performed through steps of establishing face validity (experts evaluated whether the questions effectively capture the substance of questionnaire), pilot test, reevaluation of data, principal components analysis, and internal consistency check. Index of content validity (S-CVI/UA) was 0.82. The authors collected the data using face-to-face interviews, in a private room, and those participants were then included in the study population. Completing the questionnaire took between 10-15 minutes.

Statistical Analysis

Data were provided as absolute (N) and relative (%) numbers D'Agostino-Pearson test was used for the data distribution analysis. Based on the distribution of results, a comparison between the groups was performed by the Student´s t-test for normal distribution data and Fisher exact test for categorical variables. Additionally, binary logistic regression analysis was performed. Statistical significance was defined as $P<0.05$. Statistical analyses were performed using MedCalc Statistical Sofware for Windows, version 19.0.3. (MedCalc Software, Mariakerke, Belgium).

Ethics Statement

Since no physical intervention and no information on individual health issues were involved in the study, there was no need to involve the ethical board, according to Swedish law (Swedish Health Care Act) (25). The principles of the World Medical Association Declaration of Helsinki (26) were followed carefully. The personal data were protected, i.e., names and personal identification numbers were not stated in the recordings or any publications.

Results

A total of 60 participants, 30 men, and 30 women participated in the present study. The mean age of the men and women was 41.23 and 40.49 years, respectively. Although the number of illiterate men was higher and men with a high school education were significantly lower [2 (6.7%) vs. 0 (0%), 14 (47%) vs. 22 (73%), respectively], there was no significant difference between the groups concerning the education level ($P=0.065$). Also, women had a higher income and 24 (60%) of them were unmarried. At the same time, 27 (90%) men were unmarried. All the participants in the present study were Muslims. The majority were employed with an income of between 200 000 and 300 000 Swedish kronor (SEK). However, a significant difference between genders was observed regarding the employment status only ($P=0.003$) (Table 1).

The responses concerning actual and self-perceived knowledge and opinions regarding OT and OD are presented in Table 2. In the question regarding the definition of transplantation, 14 (47%) men stated that transplantation is a medical procedure for the removal of tissue or organs from the body of a deceased person, while 29 (96%) women stated that it is a medical procedure for the removal of tissue or organs from the body of a living person ($P<0.0001$). Our result shows statistically significant differences between male and female participant´s responses and the largest difference was observed regarding the answer to the question on information about OD, where 18 (60%) of male respondents answered that they did not have sufficient information about OD and OT, while 22 (73%) female respondents stated the information they had received about OD and OT was above average ($P<0.0001$) (Table 2).

A similar ratio was found related to the self-perceived knowledge, where women indicated they had excellent knowledge about OD and OT in 20 (66.7%) of responses, while 17 (56.7%) men responded they had poor knowledge of OD and OT ($P<0.0001$). A similar difference was noticed regarding opinions about the importance of factors for OD, where 11(36.7%) men thought that the health condition of the recipient is the most important factor, while 23 (76.4%) women thought it´s a kinship with the organ donor ($P=0.0039$). Our results showed that there is no difference between informants about the implications of OD ($P=1.00$) (Table 2).

Table 1. Demographic and Clinical Characteristics of the Informants

Characteristics	Male (N; %)		Female (N; %)		P
Gender	30	100	30	100	
Age (years)					
< 25	2	6.7	2	6.7	
25-40	2	6.7	9	30.0	
41-55	8	26.7	13	43.3	1.00*
56-70	16	53.3	5	16.7	
> 70	2	6.7	1	3.3	
Education					
Illiterate	2	6.7	0	0.0	
Primary school	13	43.3	8	26.7	0.065†
High school	14	46.7	22	73.3	
Degree/above	1	3.3	0	0.0	
Marital status					
Unmarried	27	90.0	24	60.0	0.163†
Married	3	10.0	6	40.0	
Religion					
Muslim	30	100	30	100	
Other	0	0	0	0	
Employment					
Employed	19	63.3	24	80.0	
Unemployed	0	0.0	3	10.0	0.003†
Retired	10	33.3	1	3.3	
Other	1	3.3	2	6.7	
Income (SEK)					
<100,000	11	36.7	5	16.7	
100,000 - 200,000	5	16.7	6	20.0	
201,000 - 300,000	6	20,0	8	26.7	1.00*
301,000 - 500,000	7	23.3	10	33.3	
>500,000	1	3.3	1	3.3	

*Student´s t-test; †Fisher exact test; SEK=Swedish kronor.

The responses regarding the usefulness, support for, risks, and religious issues regarding OD are presented in Table 3. When asked whether they believe OD is useful and should be promoted, 16 (53.3%) men and 27 (90%) women replied that they completely agreed, while 8 (26.7%) men and two (6.7%) women said that they agreed. Although this was a statistically significant difference, the vast majority of both men and women (80% and 96.7%, respectively) were still generally in agreement that OD is useful and should be promoted more intensively. Only 5 (16.7) men and one (3.3%) woman replied "I do not agree or disagree" that OD is useful (P=0.0179) (Table 3).

Concerning the question about accepting organs from people who are of other religions, 27 (90%) women replied positively, compared to 19 (63%) men (P=0.0297). No statistically significant differences between the groups were registered in the replies to the other questions in Table 3.

Responses related to the religious aspects of OD are presented in Table 4. The result of our study showed gender differences in the responses to all the questions. The greatest difference was noticed in the responses about whether their religion permitted OD, where 21 (70%) women and 4 (13.3%) men responded that their religion allows the OD, regardless of the consequences for the person who makes the donation. Furthermore, 15 (50%) men and 3 (10%) women answered that they were not sure whether their religion allows OD (P=0.0001) (Table 4).

Table 2. Actual and Self-Perceived Knowledge and Opinions Regarding Organ Donation and Transplantation

Question / Statement	Male (N; %)		Female (N; %)		P*
How do you define transplantation?					
As a medical procedure between a deceased and a sick person	14	46.7	1	3.3	< 0.0001
As a medical procedure between a living and a sick person	7	23.3	29	96.7	
As a medical procedure between a living or a deceased person to a sick person	7	23.3	0	0.0	
As a medical procedure without reason	14	46.7	1	3.3	
Have you heard of organ transplantation?					
Yes	28	93.3	28	93.3	0.388
No	2	6.7	2	6.7	
What are the most important factors in organ donation?					
The age of the recipient	9	30.0	3	10.0	0.0017
The relationship with the donor	9	30.0	23	76.7	
Health condition	11	36.7	4	13.3	
Religious affiliation	1	3.3	0	0.0	
How do you rate your knowledge regarding organ donation?					
Poor	17	56.7	1	3.3	<0.0001
Good	11	36.7	9	30.0	
Excellent	2	6.7	20	66.7	
How do you rate the information you received about organ donation?					
Insufficient	18	60.0	1	3.3	<0.0001
Satisfactory	11	36.7	7	23.3	
Above average	1	3.3	22	73.3	

*Fisher exact test.

Table 3. Attitudes Towards Usefulness, Support for, Risks, and Religious Aspect of Organ Donation

Question / Statement	Male (N; %)		Female (N; %)		P*
Organ donation is useful.					
I completely agree	16	53.3	27	90.0	0.0179
I agree	8	26.7	2	6.7	
I neither agree nor disagree	5	16.7	1	3.3	
I disagree	1	3.3	0	0.0	
I completely disagree	0	0.0	0	0.0	
Do you support organ donation?					
Yes	26	86.7	27	90.0	0.23
No	1	3.3	3	10.0	
I don't know	3	10.0	0	0.0	
Would you agree to an organ transplant if your life is in danger?					
Yes	22	73.3	27	90.0	0.2309
No	2	6.7	2	6.7	
I don't know	6	20.0	1	3.3	
Would you accept organs from a person of another religion?					
Yes	19	63.3	27	90.0	0.0297
No	3	10.0	2	6.7	
I don't know	8	26.7	1	3.3	
Would you donate organs to a person of another religion?					
Yes	21	70.0	27	90.0	0.0755
No	2	6.7	2	6.7	
I don't know	7	23.3	1	3.3	

*Fisher exact test.

Table 4. Religious Aspects of Organ Donation, Attitudes Towards Organ Donation and Donor Card Possession

Question	Male		Female		P*
Is organ donation against your religion?					
Yes	2	6.7	1	3.3	0.0001
No	15	50.0	28	93.3	
I don't know	13	43.3	1	3.3	
What are the reasons why you are against organ donation?					
Fear of manipulation	5	16.7	1	3.3	0.0006
Untouched body after death	4	13.3	18	60.0	
Religious beliefs	1	3.3	0	0.0	
I have not thought about donation.	20	66.7	11	36.7	
To whom would you donate an organ?					
Family	12	40.0	4	13.3	0.0026
Friends	2	6.7	0	0.0	
Anybody	12	40.0	25	83.3	
I won't donate	4	13.3	1	3.3	
What is the religious aspect of organ donation?					
Same religion	1	3.3	5	16.7	0.0002
Other religion	16	53.3	24	80.0	
I don't know	13	43.3	1	3.3	
Does your religion allow organ donation?					
Yes, regardless of the consequences	4	13.3	21	70.0	0.0001
Yes, if I can survive without the organ	8	26.7	6	20.0	
Yes, after death	1	3.3	0	0.0	
No, not allowed	2	6.7	0	0.0	
I am not sure	15	50.0	3	10.0	
Do you know anyone who has donated organs?					
Relatives	3	10.0	13	43.3	0.0026
Friends	5	16.7	1	3.3	
Acquaintances	2	6.7	5	16.7	
I don't know anyone who has donated	0	0.0	2	6.7	
I don't know	20	66.7	9	30.0	
Do you own a donation card?					
Yes	1	3.3	20	66.7	<0.0001
No	29	96.7	10	33.3	
What is the character of a donor card?					
Informative	24	80	29	96.7	0.1027
Binding	6	20	1	3.3	

*Fisher exact test.

Concerning the question about religious opposition to OD, 15 (50%) men and 28 (93.3%) women responded that their religion and religion, in general, did not oppose the OD, while 13 (43%) men and one (3,3%) woman indicated that they did not know the answer to this question (P=0.0007). In the question about the reasons why the informants were against OD, the answers differed concerning gender, where 20 (66.7%) men and 11 (36.7%) women had not thought about OD and 4 (13.3%) men and 18 (60%) women believed that the body should be intact after death (P=0.006). Furthermore, 25 (83.3%) women and 12 (40%) men replied that they would donate their organs to anyone, while 12 (40%) men and 4 (13.3%) women stated that they would donate to their family members (P=0.002) (Table 4).

As many as 24 (80%) women and 16 (53.3%) men replied that the person to whom they donate their organs could have different religious

affiliation, but, at the same time, 13 (43.3%) men and one (3,3%) woman stated that they had not thought about OD (P=0.0002). Moreover, 20 (66.7%) men and 9 (30%) women replied that they did not know anyone who had donated organs, while 13 (43.3%) women and three (10%) men indicated that they did know an organ donor and that this person was a relative (P=0.0062). Ownership of a donor card was registered in 20 (66.7%) women and only one man (3%) (P<0.0001). Responses about whether the donor card was an informative or a binding statement differed, where 29 (96.7%) of women and 24 (80%) men stated that the donor card was an informative statement (P=0.103) (Table 4).

Due to observed differences between males and females, binary logistic regression analysis was performed for adjusting the effect of these differences in comparing female and male attitudes toward OD. Logistic regression analysis showed that sex and income as independent values were statistically significant in prediction of positive attitudes toward OD (possession of donor card) (P=0.0027, P=0.0002, respectively), while age and education were non-significant independent values (P=0.303, P=0.135, respectively). Also, there is a positive correlation between sex and income and attitudes towards OD (r^2=0,299, r^2=0,142, respectively).

Discussion

The present study is the first of its kind in Sweden that investigates the gender differences in perception and willingness regarding OD. The questionnaire was specifically divided into sections focusing on various information about the participants, their sociodemographic characteristics, and personal beliefs.

A strong correlation between the education level and OD was observed in previous studies (11, 12). This issue is further complicated if gender differences are taken into account. The results of the present study showed that all the illiterate participants were male. The number of women with a high school education was higher compared to the men, furthermore women had higher incomes than men. When it comes to marital status, 27 (90%) men and 24 (60%) women were unmarried. These results were initially unexpected, due to the fact that traditionally in Bosnia and Herzegovina men are more often highly educated and have a higher income than women, while the ratio between married and unmarried is roughly the same. A possible explanation could be that the majority of the respondents grew up, graduated, and started their first jobs in Sweden and that, apart from inheriting the culture from their home country, they also adopted the Swedish culture and behavioral models. Our findings contrast those in other studies, which showed that men were more educated and informed than women and subsequently had more information and more knowledge about OD (27, 28).

Also, we looked at actual and self-perceived knowledge and opinions regarding OT and OD depending on gender. The present study demonstrated that men and women perceived definitions of OT differently. We found a statistically significant difference in being informed and knowing about OD between genders, where 18 (60%) men answered that they were not sufficiently informed about OD, while 22 (73%) women stated their above-average satisfaction with available information. These findings are comparable with the results of study performed by Sipkin et al., in which the majority of all the informants were adequately informed about OD (29). Knowledge relating to OD was higher in women, 66% vs. 56% respectively. Despite reported gender differences, knowledge about OD was high in both men and women. Our findings are similar to those in a mentioned study which showed that 41.5% of all informants had adequate knowledge of OD (29).

Gender differences were also notable regarding the perception of important factors related to OD. Our results showed that 11 (37%) men considered the health of the recipient´s body as the most important factor, while 26 (77%) women stated that the most important factor is the kinship of the organ donor. Our study also revealed gender differences in the perception of usefulness and support

towards OD, with 16 (53.3%) men and 27 (90%) women replied that they completely agreed, while 8 (26.7%) men and 2 (6.7%) women agreed that OD is useful and should be promoted. The social and cultural roles of men and women differ, and they expressed different opinions regarding the usefulness of and support for OD. Women generally feel more responsible and act as caregivers, and as a result, they are much more likely to donate their organs than men. In many countries, the traditional role of women also includes looking after family members when they are ill (30, 31).

Religious aspects of OD were described differently according to gender. The women had more knowledge of their religion's permissive attitude towards OD (70% vs. 13%), and the majority of women (90%) knew that their religion did not oppose the donation of organs. In the question about reasons why the informants were against OD, the answers differed between the genders. Here, 20 (67%) men and 11 (37%) women stated that they did not think about OD, and 4 (13%) men and 18 (60%) women believed that the body should be intact after death. In other studies, men were found to refuse to donate their organs after death more often than women (32), as men were more worried about their physical integrity, believing that desecrating their bodies would bring them misfortune (33). The results of our study correspond to other studies that emphasize the need for a simpler consent system where family members could not overrule their donation decision, greater public awareness for OD, and the availability of more information on the OD process (34).

Gender differences were also demonstrated in the question about the person to whom the informants would donate their organs, where 25 (83%) women and 12 (40%) men indicated that they would donate their organs to anyone, while 12 (40%) men and 4 (13%) women would donate to their family members. Motherhood may also give women a sense of duty to volunteer for OD to save their spouse, children, and other family members. Our findings are in line with those from another study which shows that sisters, mothers, and wives more frequently donate their living organs to children, brothers, fathers, and husbands (30, 35). An interesting study showed that more than 30% of eligible wives were willing to donate their organs to their husbands, while only 7% of husbands were willing to donate their organs to their wives (36). Moreover, 24 (80%) women and 16 (53%) men were willing to donate to people with other religious affiliations and 13 (43%) men and one (3,3%) woman responded that they did not think about donating organs at all. The majority of the women did not know anyone who had donated their organs.

The results of the present study also revealed gender differences in the ownership of donor cards. In our study, 20 (66%) women owned a donor card, as opposed to only one man. The vast majority of women thought that the donor card was informative. Our findings are in line with those in previous studies, which showed that women were more willing to sign a donor card and donate their organs (32, 37). However, previous studies from low-income and under-developed countries reported results that are contrary to our findings (38, 9).

Regression analysis showed that female gender and higher income were statistically significant in prediction of positive attitudes toward OD. It is difficult to give a specific reason for a better response toward OD in women. We believe that women are more motivated, altruistic, as well as more ready to help a close family member or a person outside the family to survive. Furthermore, we shouldn't forget that motherhood and care for the family play a significant role in almost every culture of the world.

The effect of religion on the attitude towards OD becomes even more complex when gender differences are taken into account. Almost all world religions basically have a positive attitude towards OD. Islam considers OD as an expression of altruism and generosity and encourages Muslims to donate their organs (39). Some studies showed that religion is associated with a negative attitude towards OD (40). Recent survey demonstrated that even Islamic religious officials are unsure about compliance of OD and their religious belief (41).

Despite this, Gross et al. reported a positive impact of religion on attitudes towards OD. This

is especially pronounced in cases of sufficiently informed participants, who had close next of kin who were aware of their attitudes, had contacts with transplanted person, and believed in an existence after death (42). All this suggests that religious issues play a significant role and affect OD much more than we believe.

To the best of our knowledge, this is the first study of its kind among immigrants in Sweden. However, our study has some limitations, such as its cross-sectional design and the relatively small sample. This could make it difficult to generalize the findings of our study. The other limitation of the present study could be fact that the first author has the same ethnicity as the informants, which could have affected informants' responses. At the same time, Sweden remains one of the clearest examples of a multiculturalism society in Europe and a positive immigrant integration model (43). We believe that research of this kind among immigrants can contribute to their better integration into society, but also improve the necessary changes in various social aspects, including OD, in the country of origin. The experiences of other countries indicate that efforts to increase the OD rate should be focused on children and young individuals (44).

In achieving a suitable social climate for donation, gender differences may be very important, both among potential donors and transplant recipients. This can be especially important among immigrants coming from traditional societies such as Bosnia and Herzegovina. Also, it is important to identify the part of the population in which the implementation of educational measures would give the most results in the promotion of OD.

Healthcare professionals and governmental and non-governmental organizations should take the initiative actively to motivate people to give their consent and thus promote OD to a greater extent.

Conclusion

Different socioeconomic factors, cultural beliefs, a higher level of religiousness and knowledge about OD may result in a better perception of and willingness to participate in OD. Our results demonstrate how the change of social climate influences gender differences in the perception and willingness to participate in OD process, as women were found to have more positive attitudes toward this issue. Change of social background and integration into Swedish society undoubtedly led to change in the attitudes toward OD, regardless of the perspective of gender differences. Improving knowledge about all aspects of OD and the reduction in prejudice regarding these issues would make awareness and desire for OD even greater.

What Is Already Known on This Topic:

The present study deals with the public health topic of organ donation. As we know, organ donation is a life-saving concept affected by the legislation, cultural and ethnic background. Studies showed the lack of knowledge regarding this problem among the population, as one of the main reasons for inadequate acceptance of donation in public opinions. Gender differences may be very important, not just among potential donors, but also among transplant recipients.

What This Study Adds:

The present study aimed to assess gender differences, along with education level, perception, and willingness towards organ donation among Bosnian immigrants living in Sweden. The results of this study highlight the importance of achieving a suitable social climate for donation. It seems that religious and traditional concerns affect this process more than we want to believe. Subsequently, a change in the social environment can positively influence the attitudes and perception of available information. In conclusion, we can say that evaluation of this knowledge is needed to develop more efficient educational programs. To our knowledge, this is one of the first studies of its kind and can show the direction of further development of public promotion models regarding organ donation.

Ethical Approval: Not required. The consent of the Ethics Committee for conducting the research was not sought since this was a non-clinical observational study. The respondents' participation was voluntary.

Authors' Contributions: Conception and design: FK; Acquisition, analysis and interpretation of data: FK, JA and KG; Drafting the article: JA and KG; Revising it critically for important intellectual content: FK and JA; Approved final version of the manuscript: all authors.

Conflicts of interest: The authors declare that they have no conflict of interest.

References

1. Bezinover D, Saner F. Organ transplantation in the modern era. BMC Anesthesiol. 2019;19(1):32. doi: 10.1186/s12871-019-0704-z.
2. Edwards TM, Essman C, Thornton JD. Assessing racial and ethnic differences in medical student knowledge, attitudes and behaviors regarding organ donation. J Natl Med Assoc. 2007;99(2):131-7.
3. Li MT, Hillyer GC, Husain SA, Mohan S. Cultural barriers to organ donation among Chinese and Korean individuals in the United States: a systematic review. Transpl Int. 2019;32(10):1001-18. doi: 10.1111/tri.13439.
4. Demirag S. The Impact of the medical education on student's knowledge, opinion and attitude to organ/tissue donation-transplantation. Int Arch Intern Med. 2019;3:015. doi: 10.23937/2643-4466/1710015.
5. Global Observatory on Donation and Transplantation [cited 2021 Aug 18]. Available from: http://www.transplant-observatory.org.
6. International Registry In Organ Donation And Transplantation [cited 2021 Aug 18]. Available from: http://www.irodat.org.
7. Bruzzone P. Religious aspects of organ transplantation. Transplant Proc. 2008;40(4):1064-7. doi: 10.1016/j.transproceed.2008.03.049.
8. Morgan M, Hooper R, Mayblin M, Jones R. Attitudes to kidney donation and registering as a donor among ethnic groups in the UK. J Public Health (Oxf). 2006;28(3):226-34. doi: 10.1093/pubmed/fdl045.
9. Sadic S, Sadic J, Krupic R, Fatahi N, Krupic F. The influence of information and religion on organ donation, as seen by school teachers in Bosnia and Herzegovina. Mater Sociomed. 2016 Oct;28(5):373-7. doi: 10.5455/msm.2016.28.373-377.
10. Chen JX, Zhang TM, Lim FL, Wu H, Lei TF, Yeong PK, et al. Current knowledge and attitudes about organ donation and transplantation among Chinese university students. Transplant Proc. 2006;38(9):2761-5. doi: 10.1016/j.transproceed.2006.08.140.
11. Conesa C, Rios A, Ramırez P, Canteras M, Rodrıguez MM, Parrilla P. Attitudes toward organ donation in rural areas of south eastern Spain. Transplant Proc. 2006;38(3):866-8. doi: 10.1016/j.transproceed.2006.02.028.
12. Miles MS, Frauman AC. Public attitudes toward organ donation. Dial Transplant. 1988;17(2):74-6.
13. Rumsey S, Hurford DP, Cole AK. Influence of knowledge and religiousness on attitudes toward organ donation. Transplant Proc. 2003;35(8):2845-50. doi: 10.1016/j.transproceed.2003.10.078.
14. Gu Y, Dirsch O, Dahmen U, Yuan Ji, Qing He, Haidong Chi, et al. Impact of donor gender on male rat recipients of small-for-size liver grafts. Liver Transplant. 2005;11(6):669-78. doi: 10.1002/lt.20408.
15. Głyda M, Czapiewski W, Karczewski M, Pięta R, Oko A. Influence of donor and recipient gender as well as selected factors on the five-year survival of kidney graft. Pol Przegl Chir. 2011;83(4):188-95. doi: 10.2478/v10035-011-0029-1.
16. Gong W, Klöpfel M, Reutzel-Selke A, Jurisch A, Vogt K, Haase S, et al. High weight differences between donor and recipient affect early kidney graft function - a role for enhanced IL-6 signaling. Am J Transplant. 2009;9(8):1742-51. doi: 10.1111/j.1600-6143.2009.02725.x.
17. Oshakbayev K, Kuttymuratov G, Ospanova A. Causes of shortage of living/cadaveric organs and tissues donation: results of a public poll in Kazakhstan. Medical and Health Science Journal. 2012;10(5):67-73.
18. Biller-Andorno N. Gender imbalance in living organ donation. Med Health Care Philos. 2002;5(2):199-204. doi: 10.1023/a:1016053024671.
19. Legato, MJ. Gender-specific issues in organ Transplantation. In: Sanfey IH, Frcsi F, editors Principles of gender-specific medicine. 1st ed. New Work: Academic Press; 2004. p. 1116-27.
20. Weiss ES, Allen JG, Patel ND. The impact of donor-recipient sex matching on survival after orthotopic heart transplantation: analysis of 18 000 transplants in the modern era. Circ Heart Fail. 2009;2(5):401-8.
21. Minambres E, Llorca J, Subrviola B, Ortiz-Melón F, González-Castro A. Influence of donor-recipient gender mismatch in early outcome after lung transplantation. Transpalnt Proc. 2008;40(9):3076-8.
22. Zeier M, Dohler B, Opelz G, Ritz E. The effect of donor gender on graft survival. J Am Soc Nephrol. 2002;13(10):2570-6.
23. Weisdorf D, Hakke R, Blazar B, W Miller W, McGlave P. Risk factors for acute graft-versus-host disease in histocompatible donor bone marrow transplantation. Transplantation. 1991;51(6):1197-203.
24. Steinman JL. Gender disparity in organ donation. Gend Med. 2006;3(4):246-52.
25. Swedish Health Care Act. The Act concerning the ethical review of research involving humans. [cited 2015 Jun 20]. Available from: http://www.epn.se/eng/start/2003_460.apsx.
26. The World Medical Association Declaration of Helsinki. Code of Ethics 1964 (revised). Edinburgh: World Medical Association; 2005.
27. Georgiadou E, Sounidakis N, Mouloudi E, Giaglis P, Giasnetsova T, Marmanidou K, et al. Attitudes and behavior toward organ donation in Greece. Transplant Proc. 2012;44(9):2698-701. doi: 10.1016/j.transproceed.2012.09.017.
28. Vijayalakshmi P, Sunitha TS, Rohini T, Suresh BM. Gender differences in perceptions and attitudes of general population towards organ donation: An Indian Perspective. Saudi J Kindey Dis Transplant. 2017;28(3):599-608.

29. Sipkin S, Sen B, Akan S, Malak AT. Organ donation and transplantation in Onsekiz Mart Faculty of Medicine, Fine Arts and Theology: Academic staff's awareness and opinions. Adnan Menderes Univ Tip Fak Derg. 2010;11(7):19-25.
30. Abdeldayem H, Kashkoush S, Hegab BS, Aziz A, Shoreem H, Saleh S. Analysis of donor motivations in living donor liver transplantation. Front Surg. 2014;1:25. doi: 10.3389/fsurg.2014.00025. Erratum in: Front Surg. 2020 Jun 30;7:34.
31. Teegen EM, Krebs I, Langelotz C, Pratschke J, Rau B. Gender mainstreaming and transplant surgery. Visc Med. 2016;32(4):286-9.
32. Decker O, Winter M, Brahler E, Beutel M. Between commodification and altruism: Gender imbalance and attitudes towards organ donation. A representative study of the German community. J Gend Stud. 2008;17(3):251-5.
33. Bresnahan M, Lee SY, Smith SW, Shearman S, Yoo JH. Reservations of the spirit: The development of a culturally sensitive spiritual beliefs scale about organ donation. Health Commun. 2007;21(1):45-54.
34. Irving MJ, Tong A, Jan S, Cass A, Chadban S. Community attitudes to deceased organ donation: A focus group study. Transplantation. 2012;93(10):1064-9.
35. Biller-Andorno N. Gender imbalance in living organ donation. Med Health Care Philos. 2002;5(2):199-204.
36. Steinman JL. Gender disparity in organ donation. Gend Med. 2006;3(4):246-52.
37. Buitrago J, Gómez S, Guerra A, Lucumí L, Romero C, Sánchez J. Evaluation of an educational, theater-based intervention on attitudes toward organ donation in Risaralda, Colombia. Colomb Med (Cali). 2013;44(1):37-41.
38. Alashek W, Ehtuish E, Elhabashi A, Emberish W, Mishra A. Reasons for unwillingness of Libyans to donate organs after death. Libyan J Med. 2009;4(3):110-3.
39. Rady MY, Verheijde JL. Islam and end-of-life organ donation. Asking the right questions. Saudi Med J. 2009;30(7):882-6.
40. Sanner MA, Hedman H, Tufveson G. Evaluation of an organ-donor-card campaign in Sweden. Clin Transplant. 1995;9(4):326-33.
41. Akbulut S, Ozer A, Firinci B, Saritas H, Demyati K, Yilmaz S. Attitudes, knowledge levels and behaviors of Islamic religious officials about organ donation in Turkey: National survey study. World J Clin Cases. 2020;8(9):1620-31. doi: 10.12998/wjcc.v8.i9.1620.
42. Gross T, Martinoli S, Spagnoli G, Badia F, Malacrida R. Attitudes and behavior of young European adults towards the donation of organs--a call for better information. Am J Transplant. 2001;1(1):74-81. doi: 10.1034/j.1600-6143.2001.010114.x.
43. Borevi K. Understanding Swedish multiculturalism. In: Kivisto P, Wahlbeck Ö, editors. Debating multiculturalism in the Nordic welfare states. Palgrave politics of identity and citizenship series. Basingstoke: Palgrave Macmillan. 2013. p. 140-69. doi: 10.1057/9781137318459_6.
44. Akbulut S, Ozer A, Gokce A, Demyati K, Saritas H, Yilmaz S. Attitudes, awareness, and knowledge levels of the Turkish adult population toward organ donation: Study of a nationwide survey. World J Clin Cases. 2020;8(11):2235-45. doi: 10.12998/wjcc.v8.i11.2235.

Original Article
Acta Medica Academica 2022;51(1):46-51
DOI: 10.5644/ama2006-124.369

Styloid Process Length Variations: An Osteological Study

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Received: 30 March 2022; **Accepted:** 27 April 2022

Abstract

Objective. The objective of this study was to study the morphometry of the styloid process of the temporal bone and the prevalence of an elongated styloid process in relation to side and gender. **Material and Method.** The present study included 200 human skulls which were procured from the rich osteological collections of the Department of Anatomy, Faculty of Medicine, University of Sarajevo. The styloid process was observed macroscopically on both sides of all the skulls and elongations, if any, were noted. The lengths of the styloid processes were measured using digital vernier calipers. The measurements were taken from the point of emergence of the process (base) up to the tip. **Results.** Out of 200 specimens, only 14 cases (7%) exhibited an elongated styloid process. The mean length of the styloid process was 25.8±4.68 mm and 24.2±4.54 mm for the right and left sides, respectively. The size of the styloid process did not different significantly between the two sides (P=0.724). The mean length of the styloid process was 24.05±3.54 mm in females and 25.95±5.68 mm in males, and the difference was statistically significant (P=0.023). **Conclusion.** The study and knowledge of the anatomical variations of the styloid process in the Bosnian population may help clinicians to diagnose Eagle's syndrome. Knowledge of this disorder can prevent the worsening of the painful symptoms related to an elongated styloid process.

Key Words: Elongated Styloid Process ▪ Anatomic Variation ▪ Eagle's Syndrome.

Introduction

The styloid process is a thin and sharp bone structure, protruding downward and forward from the underside of the temporal bone. It is situated between the internal and external carotid arteries, posterior to the pharynx, which covers the stylohyoid, styloglossus and stylopharyngeal muscles (1). It has embryonic origin in the Reichert's cartilage of the second arch, together with the stylohyoid ligament and the lesser horn of the hyoid bone forms the stylohyoid complex or stylohyoid apparatus (2). In the adult, the stylohyoid ligament, which is normally composed of dense fibrous connective tissue, may retain some of its embryonic cartilage and thus have the potential to become partially or completely ossified. If these structures solidify, they can cause the pain and suffering present in Eagle's syndrome (3).

Steinmann proposed various theories to explain ossification. These were: "The theory of reactive hyperplasia" - trauma can cause ossification at the end of the styloid process down the length of the styloid ligament, since the styloid ligament contains remnants of its connective tissue and fibrocartilaginous origins, the potential for ossification remains; "The theory of reactive metaplasia"- an abnormal post -traumatic healing response which initiates the calcification of stylohyoid ligament; and "The theory of anatomic variance" - the early elongation of the styloid process and ossification of the styloid ligament are anatomical variations that occur without recognisable trauma (4).

The normal length of the styloid process is between 25 and 30 mm, and may vary from person to person and even between the two sides of the same individual. When processes exceed this average the term "elongation" is used (5). The elongation of the styloid process is considered an anomaly which may be accompanied by calcification of the stylohyoid and stylomandibular ligaments, and this may trigger a series of symptoms such as: dysphagia, odynophagia, facial pain, ear pain, headache, tinnitus and trismus. This set of symptoms associated with an elongated styloid process is called Eagle's syndrome (6).

The first mention of the pain syndrome associated with an elongated styloid process referred to as "stylalgia" dates back to 1937, when it was described by the American otorhinolaryngologist, Watt Weems Eagle (7). In recent literature, Eagle's syndrome has also been called stylohyoid syndrome, styloid syndrome, elongated process syndrome, stylalgia, styloid–stylohyoid syndrome, styloid dysphagia, chronic styloid angina, temporal rheumatic styloiditis, stylocarotid syndrome or Garel–Bernfeld syndrome (8). Over the last decade, experts have shown a lively interest in the issue of the relationship between the elongated styloid process and various symptoms. A number of scientific papers concerning Eagle´s syndrome or elongated styloid process were published between 2000 and 2020.

On the one hand, interest in this phenomenon is surely the result of the enormous technological developments in medical imaging in the field of radiology. Nowadays, the diagnosis of the elongated styloid process is established by three-dimensional computed tomography (9) or cone beam computed tomography (10), which provide more precise topographic-anatomical and morphometric descriptions of this anatomical anomaly, compared to panoramic radiographs (11). Considering the dynamic alteration of the anatomical relationship between the elongated styloid process and surrounding nerves and vessels relative to head position, Siniscalchi recommended that both magnetic resonance imaging (MRI) and ultrasonography of the head and neck region should be performed with the head in different positions (rest position, maximum extension, maximum flexion of the head, and maximum right and maximum left rotation) (12).

On the other hand, interest in the issue of an elongated styloid process is growing among surgical experts due to expanding options in terms of surgical approaches to the resection of this anatomical anomaly. To this day, no standardized treatment algorithm for an elongated styloid process has been established, although various surgical approaches have been described. Even though the traditional approaches (transcervical or transoral styloidectomy) are still in practice, novel modalities, such as transoral robotic surgery, have been employed lately in selected patients, to avoid the potential shortcomings associated with other approaches (13).

Finally, yet importantly, it is necessary to emphasize perhaps the most significant factor in the "rise of popularity" of Eagle´s syndrome — the newly described neurological symptomatology possibly associated with an elongated styloid process. This may be a causative factor in the development of internal carotid artery compression (14), significant compression of the internal jugular vein (15, 16), stylocarotid syndrome due to mechanical irritation of the sympathetic plexus in the cervical internal carotid artery (17), or stroke due to carotid artery dissection (18, 19). These recently described neurological morbidities, possibly caused by an elongated styloid process, have a tendency to spark the interest of many other scientists, a phenomenon known as the "snowball effect"—one study builds upon the previous one. Therefore, the number of papers pointing to the importance of this anatomical variation in the pathogenesis of various conditions is growing exponentially, which in turn further enhances the substantial attractiveness of the topic, yielding high numbers of citations.

The aim of this study was to determine the frequency of styloid process elongation in a sample of the Bosnian population.

Materials and Methods

Two hundred skulls from the collection of the Department of Anatomy, Faculty of Medicine,

University of Sarajevo, were used in the present study. Of this number, 109 (54.5%) were male and 91 (45.5%) were female skulls. The ages ranged between 23 and 91 years, with a mean of 57 years (±18.24 standard deviation) for males, and between 19 and 84 years, with a mean of 51.5 years (±16.62 standard deviation) for females.

The distances between the bases and tips of the styloid processes were measured with the help of digital vernier calipers. All the measurements were performed by the authors. The apparent length of the styloid process was measured from the point of emergence of the process to its tip, regardless of whether or not the styloid process was segmented. All measurement was carried out in accordance with the Declaration of Helsinki from 1975 and its amendments from 1983.

Statistical Analysis

The 'Paired Samples *t*-test' was used to evaluate the mean differences between the measured parameters between the right and left sides. A comparison was performed between the right and left sides, and males and females, regarding the symmetrical structures, and $P<0.05$ was considered statistically significant. Statistical analysis was performed using the statistical package IBM Statistics SPSS V19.0. Mean values, standard deviation and the range were all taken into consideration in the statistical analysis.

Results

The morphometric data on the styloid process obtained in the present study is presented in Table 1. Statistical processing of the presented results showed that there is no statistically significant difference in the length of the styloid process in relation to the side ($P=0.724$), but a statistically significant difference was noted in the length of the styloid process in relation to gender ($P=0.023$).

An elongated styloid process was found in the examined material in 7% of cases. There were 5 skulls with a styloid process over 30 mm on the right side (4.6%) and 7 (6.4%) on the left side, in males (Figure 1).

There was only 1 (1.1%) skull with styloid processes over 30 mm in a female (1 on the right, and 1 on the left), (Figure 2).

Figure 1. Elongated styloid process on the right side of a male skull, lateral view (right - 46.61 mm).

Table 1. Measurement of the Styloid Processes

Styloid processes	Gender				P*
	Male		Female		
	Range	Mean±SD	Range	Mean±SD	
Right side (mm)	19.51-46.61	27.1±5.59	16.46-40.96	24.5±3.76	0.724
Left side (mm)	17.00-38.00	24.8±5.76	15.58-39.18	23.6±3.32	
P*	0.023				

SD=Standard deviation; *Paired Samples *t*-test.

Figure 2. Elongated styloid processes on both sides of a female skull, posterior view (right 40.96 mm, left 39.18 mm).

Discussion

The styloid process is part of the stylohyoid complex, along with the lesser cornua of the hyoid bone and the stylohyoid ligament. Excessive or abnormal ossification of the stylohyoid complex components during development may result in an abnormally elongated or angulated styloid process (20, 21). The length of the styloid process was studied by Wang et al., Basekim et al., Savranlar et al., and Jung et al., using radiography and three-dimensional computed tomography (22-25).

According to the literature, the normal length of the styloid process is between 20-30 mm, but it can vary from person to person and even between the two sides of the same individual (26). There is no consensus among researchers as to which length of styloid process should be called elongated. Jung et al. suggested that the styloid process should be considered to be elongated when its length exceeds 45 mm, (24). Keur et al. stated that if the length of the process or the mineralised part of ligaments which appeared on radiography was 30 mm or more, this could be considered an elongated styloid process (4). These different results may be due to the use of different methods for measuring the styloid process. Some authors suggest that measuring the styloid process using plain bones gives the best results compared to radiographs, but data on the osteometric values of the styloid process are scanty. Previous research has shown that the length of the styloid process varies significantly between different populations, ethnic groups and geographical origin.

Our study, based on morphometric analysis of styloid processes in Bosnian skulls, showed that there was no statistically significant difference in the length of the processes on the right and left sides (P=0.724). The average length of the styloid process recorded on the right side was 25.8 ± 4.68 mm, and 24.2±4.54 mm on the left side. Similar values were noted in the studies by Andreade et al., Ramadan et al., and Gozil et al. (27-29).

A statistically significant difference was recorded in our work after processing the data on the length of the styloid process in male and female skulls (P=0.023). Higher values were recorded in male compared to female skulls (25.95±5.68 mm versus 24.02±4.54 mm). Confirmation of these results was found in previous studies (25, 29, 30), although this data contradicts the data that Eagel's syndrome is more common in women.

Elongated styloid continuation was noted in the present paper in 7% of cases, more often on the left side than on the right side. This is similar to what Kaufmans noted in his research (31). Some other researchers have reported a significantly higher incidence of elongated styloid process, such as Monsuor et al., who recorded this phenomenon in as many as 21% of cases (5).

The scarce literature data on the length of the styloid process in the Bosnian population, the incidence of prolonged styloid process, the incidence of Eagel's syndrome, and other data give rise to the need for a much more extensive study than this. We hope that this paper will be an incentive for future researchers to make extensive clinical studies that would provide answers to the questions posed above.

Conclusion

The study and knowledge of the anatomical variations of the styloid process in a population may help clinicians to diagnose Eagle's syndrome. Knowledge of this disorder can prevent the worsening of the painful symptoms related to an elongated styloid process. We believe that this study provides additional information about the frequency of an elongated styloid process in the Bosnian population. Therefore, we consider it important to perform a careful analysis of the angulation of the styloid process and its relationship to adjacent anatomical structures through imaging studies.

What Is Already Known on This Topic:
The styloid process is a thin and sharp bone structure, protruding downward and forward from the underside of the temporal bone. It is situated between the internal and external carotid arteries, posterior to the pharynx, which covers the stylohyoid, styloglossus and stylopharyngeal muscles. The normal length is between 25 and 30mm, and it can vary from person to person, and even between the two sides of the same individual. The elongation of the styloid process is considered an anomaly which may be accompanied by calcification of the stylohyoid and stylomandibular ligaments, and this may trigger a series of symptoms such as dysphagia, odynophagia, facial pain, ear pain, headache, tinnitus and trismus. This set of symptoms associated with an elongated styloid process is called Eagle's syndrome.

What This Study Adds:
We believe that this study provides additional information about the frequency of elongated styloid processes in the Bosnian population. The knowledge of anomalies of the styloid process may be beneficial to otorhinolaryngological surgeons (ENT surgeons), neurologists, and radiologists in daily clinical practice for proper diagnosis and treatment of Eagle's syndrome.

Acknowledgement: We thank all those who donated their bodies for scientific purposes and made it possible to make the presented anatomical research.

Authors' Contributions: Conception and design: EK and AV; Acquisition, analysis and interpretation of data: EK, AV and MŠ; Drafting the article: EK, AV, AŠ and AA; Revising it critically for important intellectual content: EK, AV and MŠ; Approved final version of the manuscript: EK, AV, MŠ, AŠ and AA.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Gray H. Anatomy descriptive and applied. Longmans, Green and Company, London, England; 1977.
2. Gray H, Standring S, Ellis H, Berkovitz BKB. Gray's anatomy: the anatomical basis of clinical practice. 39th ed. Skull and Mandible. Edinburgh; New York: Elsevier Churchill Livingstone; 2005.
3. Sá ACD, Zardo M, Paes Júnior AJO, Souza RP, Barros Neto F, Dreweck MO, et al. Elongated styloid process (Eagle syndrome): a case report [in Portuguese]. Radiol Bras. 2004;37(5):385-7. doi: https://doi.org/10.1590/S0100-39842004000500015.
4. Keur JJ, Campbell JP, McCarthy JF, Ralph WJ. The clinical significance of the elongated styloid process. Oral

Surg Oral Med Oral Pathol. 1986;61(4):399-404. doi: 10.1016/0030-4220(86)90426-3.

5. Monsour PA, Young WG. Variability of the styloid process and stylohyoid ligament in panoramic radiographs. Oral Surg Oral Med Oral Pathol. 1986;61(5):522-6. doi: 10.1016/0030-4220(86)90399-3.
6. Steinmann EP. Styloid syndrome in absence of an elongated process. Acta Otolaryngol. 1968;66(4):347-56. doi: 10.3109/00016486809126301.
7. Pinto PRO, Vieira GL, Menezes LM, Rizzatto SMD, Brücker M. Evaluation of the styloid process in subjects with Class III malocclusion [in Portuguese]. Rev Odonto Ciênc. 2008;23(1):44-7.
8. Eagle WW. Elongated styloid process; further observations and a new syndrome. Arch Otolaryngol (1925). 1948;47(5):630-40. doi: 10.1001/archotol.1948.00690030654006.
9. Montalbetti L, Ferrandi D, Pergami P, Savoldi F. Elongated styloid process and Eagle's syndrome. Cephalalgia. 1995;15(2):80-93. doi: 10.1046/j.1468-2982.1995.015002080.x.
10. Ayyildiz VA, Senel FA, Dursun A, Ozturk K. Morphometric examination of the styloid process by 3D-CT in patients with Eagle syndrome. Eur Arch Otorhinolaryngol. 2019;276(12):3453-9. doi: 10.1007/s00405-019-05602-6.
11. Şahin O, Kalabalik F, Tatar B, Odabaşi O. Cone-Beam Computed Tomographic Evaluation of Styloid Process in Patients With Temporomandibular Disorders and Asymptomatic Individuals. J Craniofac Surg. 2019;30(7):2236-8. doi: 10.1097/SCS.0000000000005979.
12. Bruno G, De Stefani A, Barone M, Costa G, Saccomanno S, Gracco A. The validity of panoramic radiograph as a diagnostic method for elongated styloid process: A systematic review. Cranio. 2022;40(1):33-40. doi: 10.1080/08869634.2019.1665228.
13. Siniscalchi EN. Dynamic imaging in suspected Eagle syndrome. Eur Arch Otorhinolaryngol. 2020;277(1):307. doi: 10.1007/s00405-019-05678-0.
14. Fitzpatrick TH 4th, Lovin BD, Magister MJ, Waltonen JD, Browne JD, Sullivan CA. Surgical management of Eagle syndrome: A 17-year experience with open and transoral robotic styloidectomy. Am J Otolaryngol. 2020;41(2):102324. doi: 10.1016/j.amjoto.2019.102324.
15. Galletta K, Granata F, Longo M, Alafaci C, De Ponte FS, Squillaci D, et al. An unusual internal carotid artery compression as a possible cause of Eagle syndrome - A novel hypothesis and an innovative surgical technique. Surg Neurol Int. 2019;10:174. doi: 10.25259/SNI_317_2019.
16. Zamboni P, Scerrati A, Menegatti E, Galeotti R, Lapparelli M, Traina L, et al. The eagle jugular syndrome. BMC Neurol. 2019;19(1):333. doi: 10.1186/s12883-019-1572-3.
17. Zhang FL, Zhou HW, Guo ZN, Yang Y. Eagle Syndrome as a Cause of Cerebral Venous Sinus Thrombosis. Can J Neurol Sci. 2019;46(3):344-5. doi: 10.1017/cjn.2019.17.
18. Eraslan C, Ozer MA, Govsa F, Alagoz AK, Calli C. Relationship of stylohyoid chain and cervical internal carotid artery detected by 3D angiography. Surg Radiol Anat. 2017;39(8):897-904. doi: 10.1007/s00276-017-1812-4.
19. Shindo T, Ito M, Matsumoto J, Miki K, Fujihara F, Terasaka S, et al. A Case of Juvenile Stroke due to Carotid Artery Dissection from an Elongated Styloid Process-Revisiting Conservative Management. J Stroke Cerebrovasc Dis. 2019;28(10):104307. doi: 10.1016/j.jstrokecerebrovasdis.2019.104307.
20. McGinnis Jr JM. Fractures of an ossified stylohyoid bone. Arch. Otolaryngol. 1981;107(7):460.
21. Camarda AJ, Deschamps C, Forest D. I. Stylohyoid chain ossification: a discussion of etiology. Oral Surg Oral Med Oral Pathol. 1989;67(5):508-14. doi: 10.1016/0030-4220(89)90264-8.
22. Wang Z, Liu Q, Cui Y, Gao Q, Liu L. Clinical evaluation of the styloid process by plain radiographs and three-dimensional computed tomography [in Chinese]. Lin Chuang Er Bi Yan Hou Ke Za Zhi. 2006;20(2):60-3.
23. Başekim CC, Mutlu H, Güngör A, Silit E, Pekkafali Z, Kutlay M, et al. Evaluation of styloid process by three-dimensional computed tomography. Eur Radiol. 2005;15(1):134-9. doi: 10.1007/s00330-004-2354-9.
24. Savranlar A, Uzun L, Uğur MB, Ozer T. Three-dimensional CT of Eagle's syndrome. Diagn Interv Radiol. 2005;11(4):206-9.
25. Jung T, Tschernitschek H, Hippen H, Schneider B, Borchers L. Elongated styloid process: when is it really elongated? Dentomaxillofac Radiol. 2004;33(2):119-24. doi: 10.1259/dmfr/13491574.
26. Eagle WW. The symptoms, diagnosis and treatment of the elongated styloid process. Am Surg. 1962;28:1-5.
27. de Andrade KM, Rodrigues CA, Watanabe PC, Mazzetto MO. Styloid process elongation and calcification in subjects with tmd: clinical and radiographic aspects. Braz Dent J. 2012;23(4):443-50. doi: 10.1590/s0103-64402012000400023.
28. Ramadan SU, Gokharman D, Tunçbilek I, Kacar M, Koşar P, Kosar U. Assessment of the stylohoid chain by 3D-CT. Surg Radiol Anat. 2007;29(7):583-8. doi: 10.1007/s00276-007-0239-8.
29. Gözil R, Yener N, Calgüner E, Araç M, Tunç E, Bahcelioğlu M. Morphological characteristics of styloid process evaluated by computerized axial tomography. Ann Anat. 2001;183(6):527-35. doi: 10.1016/S0940-9602(01)80060-1.
30. Aral IL, Karaca I, Güngör N. Eagle's syndrome masquerading as pain of dental origin. Case report. Aust Dent J. 1997;42(1):18-9. doi: 10.1111/j.1834-7819.1997.tb00090.x.
31. Kaufman SM, Elzay RP, Irish EF. Styloid process variation. Radiologic and clinical study. Arch Otolaryngol. 1970;91(5):460-3. doi: 10.1001/archotol.1970.00770040654013.

Original Article
Acta Medica Academica 2022;51(1):52-58
DOI: 10.5644/ama2006-124.370

Anatomic Variation of the Sciatic Nerve: A Study on the Prevalence, and Bifurcation Loci in Relation to the Piriformis and Popliteal Fossa

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Received: 19 July 2021; **Accepted:** 12 March 2022

Abstract

Objective. To examine and identify sciatic nerve variations in relation to the piriformis muscle, its prevalence, pattern and the course of its bifurcation loci. **Materials and Methods.** Twenty-eight formalin fixed male cadavers comprising 56 lower limbs were used for this study. Dissection of the gluteal region and posterior compartment of the thigh was conducted to expose the sciatic nerve. Variations in the sciatic nerve anatomy, their relationship to the piriformis muscle and points of bifurcation, and other observable features were noted and recorded. **Results.** Fifty-two lower limbs (93%) showed normal anatomy of the sciatic nerve. Four regions (7.1%) showed variations in the morphology of the sciatic nerve. Of these, one (1.8%) showed a variation of the sciatic nerve with the piriformis muscle. This single case showed a common peroneal nerve emerging on the left between the heads of a double piriformis muscle - a variant not described in the original Beaton and Anson classification, with the tibial nerve deep to the muscle. In two other limbs, the sciatic nerves showed a normal relationship with the piriformis, but had variations in the bifurcation loci (bilateral). The divisions were in upper third and middle third of the right and left thighs respectively. **Conclusion.** Knowledge of the level of bifurcation and distribution of the sciatic nerve and its location is important. This nerve is commonly encountered by neurologists, orthopaedics, and anaesthesiologists. The uncommon anatomical findings described are relevant to surgeons to enable them to perform efficient surgical procedures and avoid errors.

Key Words: Sciatic Nerve ▪ Bifurcation Loci ▪ Piriformis ▪ Sciatica.

Introduction

The sciatic nerve is the largest nerve in the human body, formed by the union of five nerve roots (L4-S3) arising from the lumbosacral. It carries two nerve components, namely: the tibial component (L4, 5, S1, S2, and S3) and the common-peroneal component (L4, 5, S1, and S2) (1). This nerve, in normal anatomy, enters the gluteal region from the pelvis through the greater sciatic foramen, passing inferior to the piriformis muscle. It follows a descending path along the posterior thigh, up to the proximal region of the popliteal fossa, where it divides into its terminal branches: the tibial nerve and the common peroneal nerve. The detailed anatomy of the sciatic nerve has been described elsewhere (2). However, variations from the classical descriptions have been observed concerning the location where the nerve divides, as well as its relationship to the piriformis muscle (3).

Reports suggest that certain variations of the sciatic nerve, as it emerges into the gluteal region, may be the cause of sciatica and piriformis syndrome (4, 5). Six per cent of low back pain cases are caused by piriformis syndrome, a painful condition that mimics sciatica and is ancillary to sciatic nerve entrapment (6, 7). It was also suggested that incomplete sciatic nerve block during popliteal block anaesthesia could be caused by the sciatic nerve terminating high-up or in the proximal thigh (8). To avoid iatrogenic injuries caused by clinical procedures, such as hip arthroplasty or pelvic surgery, clinicians need detailed knowledge of the anatomical variations of the sciatic nerve

and piriformis muscle (9, 10). Spinal degenerative disc disorders or spinal radiculopathies can cause sciatica, although piriformis syndrome is responsible for up to 6-8% cases of sciatica (11). Studies suggest that pain in the buttock or posterior hip, arising from non-discogenic or extra-pelvic entrapment of the sciatic nerve, may indicate piriformis syndrome (12).

Hence, the purpose of this present study was to examine sciatic nerve variations with the piriformis muscle, its pattern and course of bifurcation loci, in the black population of sub-Saharan Africa. Beaton and Anson's classification method for sciatic nerve variation with the piriformis (12) was employed in this study, just as it was previously used by several others (13, 14).

Materials and Method

Fifty-six lower limbs from 28 formalin fixed male cadavers, without any clinical data of gross pathology, were examined during routine dissection in the Gross Anatomy Laboratory of the Niger Delta University, Wilberforce Island, Bayelsa State. The gluteal regions of these cadavers were carefully dissected and the gluteus maximus muscle retracted to expose the piriformis muscle and sciatic nerve.

The posterior compartment of the thigh was also dissected, and the long head of the biceps femoris was separated from the semitendinosus muscle, to further expose the course of the sciatic nerve in the posterior compartment of the thigh. The sciatic nerve variation pattern in relation to the piriformis, the frequency and distribution of sciatic nerve bifurcation loci were examined, identified, recorded, and photographed. Data were collected for a period spanning five years. Anatomical variations were identified and classified on the basis of the Beaton and Anson classification system (12), which categorises structural variations of the sciatic nerve with the piriformis muscle in the gluteal region into six (6) types (Figure 1).

a) Type-1: undivided nerve below undivided muscle
b) Type-2: divisions of nerve between and below undivided muscle

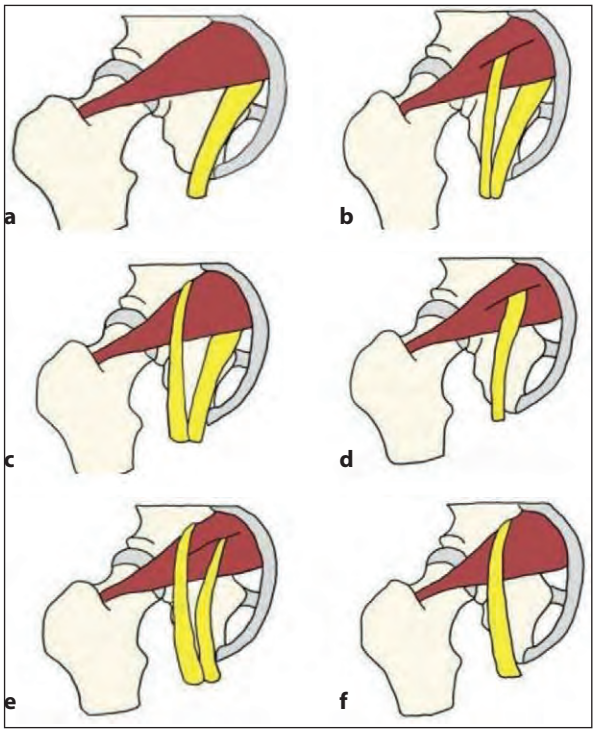

Figure 1. (a – f): Anatomic variations of the relationship between the piriformis muscle and sciatic nerve. Diagrams illustrate the six variants originally described by Beaton and Anson (a) An undivided nerve comes out below the piriformis muscle (normal course); (b) A divided sciatic nerve passing through and below the piriformis muscle; (c) A divided nerve passing above and below an undivided muscle; (d) An undivided sciatic nerve passing through the piriformis muscle; (e) A divided nerve passing through and above the muscle heads; (f) An undivided nerve above undivided muscle (15).

c) Type-3: divisions above and below undivided muscle
d) Type-4: undivided nerve between heads
e) Type-5: divisions between and above heads
f) Type-6: undivided nerve above undivided muscle

Ethical Statement

In Nigeria, government hospitals and prisons are required to release the corpses of bandits or criminals and unclaimed bodies to medical schools to aid in the training of medical students. At least 80% of all cadavers in Nigerian medical schools are from bandits or criminals that were killed in

conflicts with law enforcement agents; less than 10% are unclaimed corpses (16, 17). Once received by a department of anatomy, such bodies are available for teaching, learning, and research. By convention, cadaveric study within a gross anatomy laboratory does not require special ethical approval. Photography that may reveal identification is strictly prohibited. Respect and courtesy are observed when working with these cadavers.

Results

In this study, 52 (92.9%) out of 56 dissected lower limbs of human cadavers showed normal anatomy of the sciatic nerve, piriformis muscle, and their relationship (Figure 2), which corresponds to type-1 according to Beaton and Anson's classification (Figure 1a). Four (7.1%) limbs showed variations in the anatomy of the sciatic nerve. The most prevalent variation showed the common peroneal nerve passing through the piriformis muscle and the tibial nerve passing below. This variation is type-2 according to Beaton and Anson's classification. The two (3.6%) lower limbs of a particular cadaver showed a bilateral variation of the sciatic nerve in relation to the piriformis muscle: on the right limb, the common peroneal nerve emerged through the piriformis and the tibial nerve below, with both descending distinctly. On the left limb, the common peroneal nerve emerged between the heads of a double piriformis (double head piriformis), while the tibial nerve passed beneath the muscle (Figure 3). In both extremities in another cadaver (3.6%, bilateral), the sciatic nerve bifurcation occurred in the thigh at different locations in the right and left thighs. On the left, it was at the mid-thigh, and on the right at the upper one–third of the thigh, with a rare communicating twig connecting the common peroneal nerve to the tibial nerve (Figure 4).

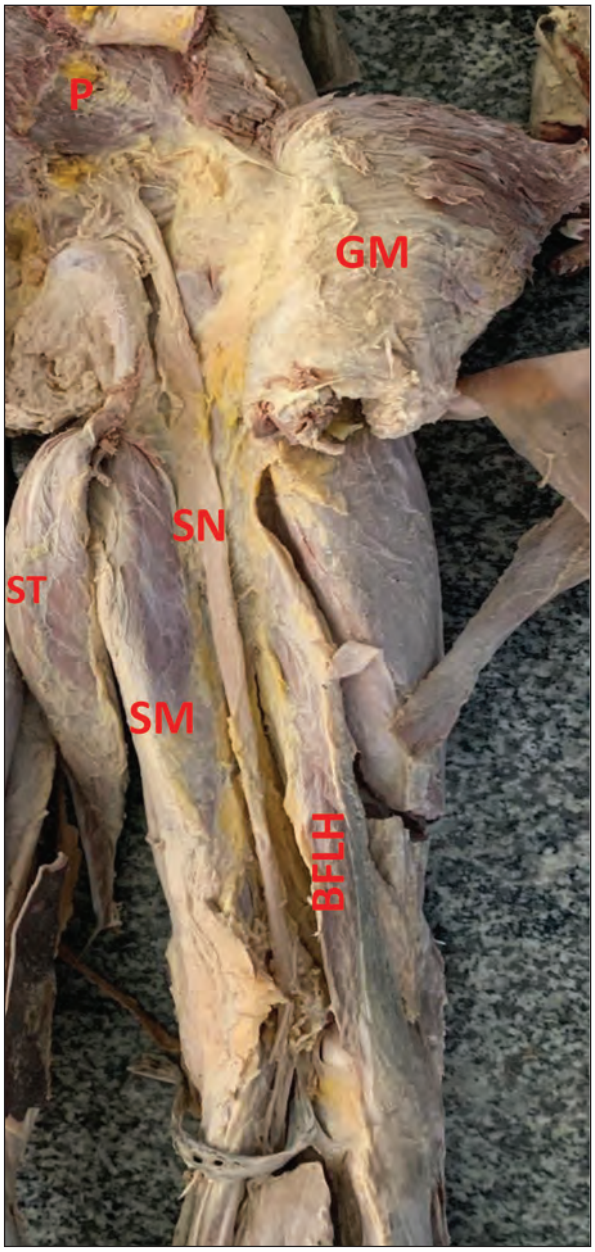

Figure 2. Picture of a dissected right lower limb showing the normal anatomy of the piriformis muscle (P); Gluteus maximus (GM); Sciatic nerve (SN); Semitendinosus (ST); Semimembranosus (SM); Biceps femoris (long head) (BFLH).

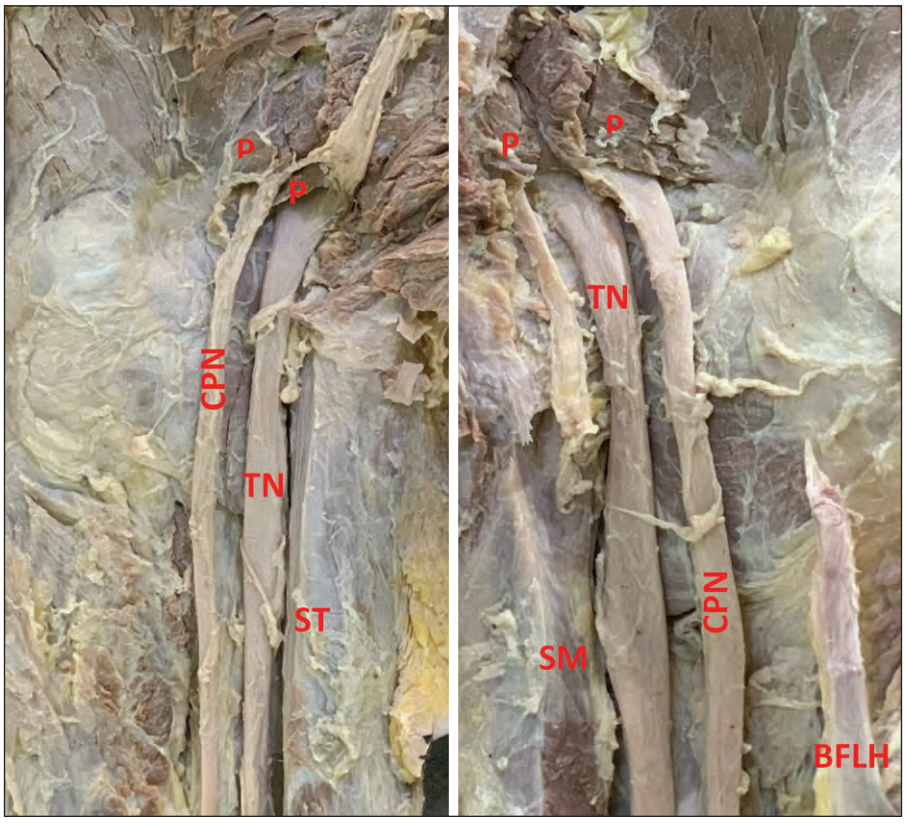

Figure 3. A bilateral variation of the sciatic nerve in relation to the piriformis muscle. A) Left lower limb showing divided piriformis muscle (P), with the common peroneal nerve (CPN) passing through and the tibial nerve (TN) below it, and both nerves descending separately. Semitendinosus (ST). B) Right lower limb showing the piriformis muscle (P), with the common peroneal nerve passing through and the tibial nerve below the muscle. Both nerves descend separately. Semimembranosus (SM), Biceps femoris (long head) (BFLH).

Table 1. Relationship between the Sciatic Nerve and Piriformis Muscle, Reported in a Previous Study Based on Beaton and Anson's Classification

Publications	Type-1 (%)	Type 2 (%)	Type-3 (%)	Type-4 (%)	Type-5 (%)	Type-6 (%)	*Type-7 (%)
Beaton (18)	90	7.1	2.1	0.8			
Beaton & Anson (12)	84.2	11.7	3.3	0.8	-	-	-
Berihu & Debeb (19)	75	11	2	-	-	-	-
Chiba (3)	66	34	-	-	-	-	-
Guvencer et al. (20)	76	16	8	-	-	-	-
Lewis et al. (21)	89	8.8	2.9	-	-	-	-
Machado et al. (22)	82	16	2	-	-	-	-
Ozaki et al. (23)	-	-	-	-	-	*One case	
Pecina (24)	93.1	6.15	-	-	-	-	-
Pokorny et al. (25)	79.1	14.3	4.4	2.2	-	-	-
Singh & Sharma (26)	96	4	-	-	-	-	-
Ugrenovic et al. (27)	96	2.5	1.5	-	-	-	-
Uluutku & Kurtoglu (28)	74	16	10	-	-	-	-
Present study	93	3.5	-	-	-	-	†3.5

*A case report; †Not included in the Beaton and Anson classification.

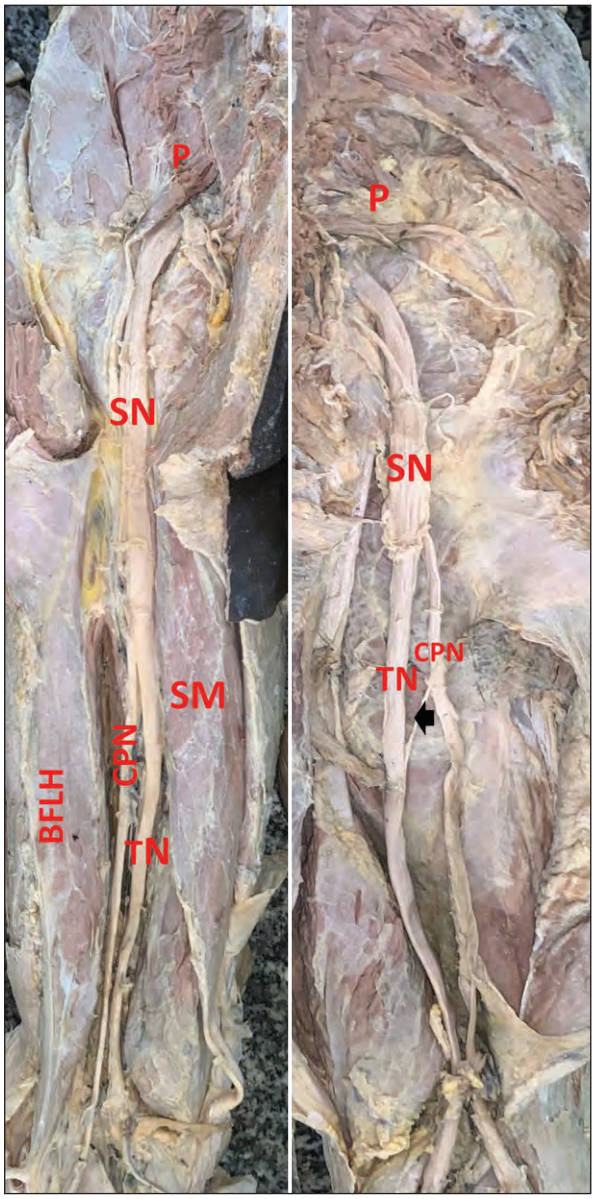

Figure 4. Bilateral sciatic nerve bifurcation variability in the thigh. A) Left lower limb showing undivided piriformis (P), with the sciatic nerve (SN) bifurcation into common peroneal nerve (CPN) and tibial nerve (TN) at the middle of the thigh. Semimembranosus (SM). B) Right lower limb showing undivided piriformis (P), sciatic nerve (SN) division into the common peroneal nerve (CPN) and tibial nerve (TN) in the upper one – third of the thigh, and a rare communicating twig (black arrow head) connecting the common peroneal nerve to the tibial nerve.

Discussion

In this study, we identified 92.9% of lower limbs with normal anatomy of the sciatic nerve in relation to the piriformis muscle, which corresponds to type 1 classification (Figure 1a). In two (3.6%) lower extremities of a particular cadaver, the common peroneal nerve and the tibial nerve were seen passing respectively through and below the piriformis muscle; this is a type-2 variant of the Beaton and Anson's classification and is the most widely reported variation, where the nerves descend separately throughout their course (3, 12). Nevertheless, there are ethnic or regional variations in sciatic nerve anatomy (3, 11, 18-28). Studies with Beaton and Anson's classification (11) show that the types 1 and 2 variations are seen in all human populations (Table 1). Types 1, 2, 3, and 4 were recorded among Americans (11) and Eastern Europeans (25). However, a similar study in an Eastern European population observed only types 1, 2, and 3 (25). Reports suggested that types 1 and 2 were the predominant variations among Indians (26) and Japanese (3, 28) respectively. A study conducted amongst the Ethiopians of sub-Saharan Africa suggested that types 4, 5, and 6 are rare, with only types 1, 2 and 3 observed (19). However, in the present work, we did not see the type-3 of the Beaton and Anson's classification among our study population (sub-Saharan Africa).

We also observed bilateral bifurcations of the sciatic nerves in the proximal half of the thighs in one cadaver, involving 2 (3.6%) limbs. The sciatic nerve bifurcates at the level of the upper third of the thigh on the right and the mid-thigh on the left. This observation suggests that the sciatic nerve can divide into its terminal divisions at any level in the thigh, consistent with previous reports (1, 10, 14). However, a bilateral variation of the sciatic nerve to the piriformis, and the varied bifurcation loci observed in a particular cadaver, as in this study, are a rare occurrence. The double-headed piriformis muscle reported in our study does not fit into the 6-type classification; it occupies a separate class. Although this variant was not anticipated by Beaton and Anson, they hypothesised

the possibility of two other types: one in which the nerve passes between and above the heads, and in the other, an undivided nerve passes above the undivided muscle (12). However, a more recent review article identified twelve variants, that is, seven additional variants to the Beaton and Anson variants (29). This suggests the possibility of a yet to be observed variation pattern. Interestingly, Barbosa et al. in their review article mentioned the occurrence of a rare variant where the sciatic nerve emerges divided, and the common peroneal nerve passes between the heads of a double piriformis, while the tibial nerve passes beneath the muscle (29). From this present study, we opined that knowledge of these anatomical variations might be useful in choosing the correct treatment for patients with piriformis syndrome or sciatica, as previously suggested (28, 30). This study highlights certain sciatic nerve variations around the gluteal region and its bifurcation loci in the thigh in a Nigerian population. It may be necessary to study the embryological basis of these anatomical variations to gain better understanding.

Conclusion

The location, bifurcation, and distribution of the sciatic nerve are of clinical importance. The long course of the sciatic nerve makes it vulnerable to injury as it is commonly involved in regular medical practices such as anaesthesia, rehabilitation, orthopaedics and neurology. Despite our findings that 7.1% of sciatic nerves have irregular anatomy, it is worth noting that none of the variations compromises intramuscular injection in the gluteal area.

What Is Already Known on This Topic:

The sciatic nerve is the thickest nerve in the human body, and the most important nerve for normal gait. It is documented in the literature that over 70% of sciatic nerves leave the pelvis undivided through the infra piriformis portion of the greater sciatic foramen, run down the posterior thigh and bifurcate in the popliteal fossa, as observed in this present study. To date, the term "idiopathic sciatica" is still in use. It is reasonable to speculate that an unknown variation from the normal anatomy of the sciatic nerve may be partly responsible for unexplainable sciatica.

What This Study Adds:

The possibility that intractable sciatica may be linked to unknown sciatic nerve variations justifies our study of its relationship with the piriformis muscle and bifurcation loci along its course. This study documents a common left side, peroneal nerve emerging between a double-headed piriformis muscle as an apparently unknown variation, not described in the original Beaton and Anson classification. We also report a rare communicating nerve connecting the common peroneal nerve with the tibial nerve near the mid-thigh. To our knowledge, these are two rare anatomical events.

Acknowledgement: The authors are grateful to the management of Agbor Central Hospital, Delta State, Nigeria and the management of Niger Delta University, Bayelsa State, Nigeria, for making human bodies available in the Department of Human Anatomy, Niger Delta University, for our medical school for teaching and research.

Authors' Contributions: Conception and design: ADA and CAO; Acquisition, analysis and interpretation of data: ADA, CAO and ULT; Drafting the article: ADA, CAO and DAUF; Revising it critically for important intellectual content: ADA, CAO and DAUF; Approved final version of the manuscript: ADA, CAO, DAUF and ULT.

Conflict of Interest: The authors declare that they have no conflicts of interest.

References

1. Standring S. Gray's anatomy: the anatomical basis of clinical practice. 39th ed. London: Churchill Livingstone; 2005. p. 1364-458.
2. Catala M, Kubis N. Gross anatomy and development of the peripheral nervous system. Handb Clin Neurol. 2013;115:29-41. doi: 10.1016/B978-0-444-52902-2.00003-5.
3. Chiba S. Multiple positional relationships of nerves arising from the sacral plexus to the piriformis muscle in humans [in Japanese]. Kaibogaku Zasshi. 1992;67(6):691-724.
4. Ro TH, Edmonds L. Diagnosis and Management of Piriformis Syndrome: A Rare Anatomic Variant Analyzed by Magnetic Resonance Imaging. J Clin Imaging Sci. 2018;8:6. doi: 10.4103/jcis.JCIS_58_17.
5. Natsis K, Totlis T, Konstantinidis GA, Paraskevas G, Piagkou M, Koebke J. Anatomical variations between the sciatic nerve and the piriformis muscle: a contribution to surgical anatomy in piriformis syndrome. Surg Radiol Anat. 2014;36(3):273-80. doi: 10.1007/s00276-013-1180-7.
6. Mitra SR, Roy S, Dutta AS, Ghosh A, Roy R, Jha AK. Piriformis syndrome: a review. Journal of Evolution of Medical and Dental Sciences. 2014;3(14):3804-14.

7. Yeoman W. The relation of arthritis of the sacro-iliac joint to sciatica, with an analysis of 100 cases. Lancet. 1928;212(5492):1119-23.
8. Kirschner JS, Foye PM, Cole JL. Piriformis syndrome, diagnosis and treatment. Muscle Nerve. 2009;40(1):10-8. doi: 10.1002/mus.21318.
9. Tomaszewski KA, Graves MJ, Henry BM, Popieluszko P, Roy J, Pękala PA, Hsieh WC, Vikse J, Walocha JA. Surgical anatomy of the sciatic nerve: A meta-analysis. J Orthop Res. 2016;34(10):1820-7. doi: 10.1002/jor.23186.
10. Prakash, Bhardwaj AK, Devi MN, Sridevi NS, Rao PK, Singh G. Sciatic nerve division: a cadaver study in the Indian population and review of the literature. Singapore Med J. 2010;51(9):721-3.
11. Jankovic D, Peng P, van Zundert A. Brief review: piriformis syndrome: etiology, diagnosis, and management. Can J Anaesth. 2013;60(10):1003-12. doi: 10.1007/s12630-013-0009-5.
12. Beaton LE, Anson BJ. The relation of the sciatic nerve and its subdivisions to the Piriformis muscle. Anat Rec. 1937;70(1):1-5.
13. Lewis S, Jurak J, Lee C, Lewis R, Gest T. Anatomical variations of the sciatic nerve, in relation to the piriformis muscle. Translational Research in Anatomy. 2016;5:15-9.
14. Anbumani TL, Thamarai SA, Anthony AS. Sciatic nerve and its variations: an anatomical study. Int J Anat Res. 2015;3(2):1121-7.
15. Kim HJ, Lee SY, Park HJ, Kim KW, Lee YT. Accessory Belly of the Piriformis Muscle as a Cause of Piriformis Syndrome: A Case Report with Magnetic Resonance Imaging and Magnetic Resonance Neurography Imaging Findings. Investigative Magnetic Resonance Imaging. 2019;23(2):142-7. doi.org/10.13104/imri.2019.23.2.142
16. Anyanwu GE, Udemezue OO, Obikili EN. Dark age of sourcing cadavers in developing countries: a Nigerian survey. Clin Anat. 2011;24(7):831-6. doi: 10.1002/ca.21187.
17. Gangata H, Ntaba P, Akol P, Louw G. The reliance on unclaimed cadavers for anatomical teaching by medical schools in Africa. Anat Sci Educ. 2010;3(4):174-83. doi: 10.1002/ase.157.
18. Beaton LE. The sciatic nerve and piriform muscle: their interrelations possible cause of coccgodynia. J Bone Joint Surg Am. 1938;20:686-8
19. Berihu BA, Debeb YG. Anatomical variation in bifurcation and trifurcations of sciatic nerve and its clinical implications: in selected university in Ethiopia. BMC Res Notes. 2015;8:633. doi: 10.1186/s13104-015-1626-6.
20. Güvençer M, Iyem C, Akyer P, Tetik S, Naderi S. Variations in the high division of the sciatic nerve and relationship between the sciatic nerve and the piriformis. Turk Neurosurg. 2009;19(2):139-44.
21. Lewis S, Jurak J, Lee C, Lewis R, Gest T. Anatomical variations of the sciatic nerve, in relation to the piriformis muscle. Translational Research in Anatomy. 2016;5:15-9.
22. Machado FA, Babinski MA, Brazil FB, Favorito LA, Abidu-Figueiredo M, Costa MG. Anatomical variations between sciatic nerve and piriform muscle during fetal period in human. Int J Morphol. 2003;21(1):29-35.
23. Ozaki S, Hamabe T, Muro T. Piriformis syndrome resulting from an anomalous relationship between the sciatic nerve and piriformis muscle. Orthopedics. 1999;22(8):771-2. doi: 10.3928/0147-7447-19990801-09.
24. Pećina M. Contribution to the etiological explanation of the piriformis syndrome. Acta Anat (Basel). 1979;105(2):181-7.
25. Pokorny D, Jahoda D, Veigl D, Pinskerova V, Sosna A. Topographic variations of the relationship of the sciatic nerve and the piriformis muscle and its relevance to palsy after total hip arthroplasty. Surg Radiol Anat. 2006;28(1):88-91.
26. Singh AK, Sharma RC. Relationship between the sciatic nerve and piriformis muscle. Neuroscience Research Letters. 2011;2(1):26-8.
27. Ugrenovic S, Jovanović I, Krstić V, Stojanović V, Vasović L, Antić S, et al. The level of the sciatic nerve division and its relations to the piriform muscle. Vojnosanit Pregl. 2005;62(1):45-9.
28. Uluutku MH, Kurtoglu Z. Variations of nerves located in deep gluteal region. Okajimas Folia Anat Jpn. 1999;76(5):273-6.
29. Barbosa ABM, Santos PVD, Targino VA, Silva NA, Silva YCM, Gomes FB, et al. Sciatic nerve and its variations: is it possible to associate them with piriformis syndrome? Arq Neuropsiquiatr. 2019;77(9):646-653. doi: 10.1590/0004-282X20190093.
30. Gulledge BM, Marcellin-Little DJ, Levine D, Tillman L, Harrysson OL, Osborne JA, et al. Comparison of two stretching methods and optimization of stretching protocol for the piriformis muscle. Med Eng Phys. 2014;36(2):212-8. doi: 10.1016/j.medengphy.2013.10.016.

Case Report
Acta Medica Academica 2022;51(1):59-63
DOI: 10.5644/ama2006-124.371

Emphysematous Pyelonephritis with IgA-Dominant Infection-Related Glomerulonephritis: An Unusual Picture

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Received: 25 January 2022; **Accepted:** 24 April 2022

Abstract

Objective. The aim of this case report is to illustrate a very rare case of emphysematous pyelonephritis complicated by IgA-dominant postinfectious glomerulonephritis. **Case Report.** We report the case of a 53-year-old woman with emphysematous pyelonephritis who initially presented with unintentional weight loss for 3 months and subnephrotic range proteinuria without fever. Urinalysis revealed proteinuria, microscopic hematuria, and pyuria. A kidney biopsy was performed for suspected glomerulonephritis. The patient's right kidney biopsy was consistent with immunoglobulin A (IgA)-dominant infection-related glomerulonephritis. Abdominal computed tomography to seek the possible source of infection revealed staghorn stones obstructing dilated calyces and gas collection within the collecting system. The final diagnosis was emphysematous pyelonephritis of the left kidney complicated by IgA-dominant infection-related glomerulonephritis of the right kidney. **Conclusion.** We present an atypical presentation of emphysematous pyelonephritis in terms of clinical presentation (prolonged course of illness without fever) and its complications (IgA-dominant infection-related glomerulonephritis). This case study highlights the critical role of kidney biopsy in the diagnosis and the diverse clinical manifestations in clinical medicine.

Key Words: Unintentional Weight Loss ▪ Proteinuria ▪ Kidney Infection ▪ Biopsy ▪ Flank Pain.

Introduction

Emphysematous pyelonephritis (EPN) is a severe necrotizing kidney infection associated with gas formation. The typical clinical spectrum of EPN encompasses fever, flank pain, and, in severe cases, thrombocytopenia, renal dysfunction, and shock may occur (1-3). The symptom duration before diagnosis is usually <10 days (1); however, longer durations have been reported (4, 5).

We report the case of a 53-year-old woman with left EPN presenting with unintentional weight loss and subnephrotic range proteinuria without fever. To the best of our knowledge, this is the first reported case of EPN presenting with a prolonged course of illness without fever and complicated by immunoglobulin (Ig) A-dominant infection-related glomerulonephritis (IgADIRGN).

Case Report

A 53-year-old woman with non-contributory past medical history was referred to our hospital for malaise and unintentional weight loss of 23 kg in 3 months. She did not experience fever, night sweats, or other abnormal symptoms. Her vitals at the time of presentation were as follows: temperature, 36.5 °C; blood pressure, 111/77 mmHg; heart rate, 102 beats/min; respiratory rate, 20 breaths/min; and oxygenation level, 98%. No significant

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physical findings were observed. Hematuria, proteinuria, and elevated leukocyte levels were noted on urinalysis (Table 1). Bloodwork showed normal white blood cell counts [50% neutrophils, 38% lymphocytes, no band form, 31% hematocrit, and 331×10^3/μL platelet count]. Serum blood urea nitrogen, creatinine, fasting blood sugar, HbA1c, and 24-hour urine protein levels were 10 (reference: 9.8–20.1) mg/dL, 0.65 (reference: 0.73–1.18) mg/dL, 81 (reference: 70–100) mg/dL, 5.59% (4.5–6.3%), and 2,189 (reference: <150) mg/day, respectively. Chest radiography showed no abnormal findings, and urine and blood cultures were negative.

Table 1. Complete Laboratory Analysis

Analysis	Reference range	At		
		Presentation	Nephrectomy	Two weeks*
Blood				
BUN	8.9-20.6 mg/dL	10	11	12
Serum creatinine	0.73-1.18 mg/dL	0.65	0.59	0.47
eGFR(EPI)	ml/min/1.73^2	101.7	105	113
Na	136-145 mEq/L	137	132	131
K	3.5-5.1 mEq/L	3.74	3.61	4.97
Cl	98-107 mEq/L	102	98	98
HCO3	22-29 mEq/L	26	22	27
WBC	4.5-10.0×10^3/μL	5.3	7.09	8.6
Neutrophil	20-50%	50	70	69
Lymphocyte	20-40%	38	21	21
Hemoglobin	12.0-16.0 g/dL	10.0	9.9	11.4
Hematocrit	37-47%	31	30.1	34
Platelet	150-450×10^3/μL	331	435	498
TSH	0.35-4.94 uIU/mL	0.46	-	-
Morning cortisol	10-20 mcg/dL	13	-	-
AST	5-34 U/L	10	-	-
ALT	0-55 U/L	6	-	-
ALP	40-150 u/L	79	-	-
Total bilirubin	0.2-1.2 mg/dL	0.3	-	-
Fasting blood sugar	70-100 mg/dL	81	-	-
Hemoglobin A1C	4.5-6.3%	5.59	-	-
Anti-HIV	Negative	Negative	-	-
ANA	Negative	1:320	-	-
C3 complement	88-165 mg/dL	184	-	-
C4 complement	14-44 mg/dL	39.5	-	-
Urine				
Analysis	-	Color: Yellow, Specific gravity: 1.011, pH: 6.0, Protein: 2+, Glucose: negative, RBC >100/HPF, WBC: >100/HPF	-	-
24-hour protein	<150 mg/dL	2,189	1,072	256
Urine culture	-	Negative	-	-
Hemoculture	-	Negative	-	-

*After nephrectomy.

Kidney biopsy was performed due to the abnormal urine sediment and proteinuria. Preoperative ultrasonography revealed a calyceal stone in the left kidney; therefore, biopsy was performed on the right kidney. There were 17 glomeruli, including 1 with global sclerosis. Mild mesangial expansion and no endocapillary proliferation or exudative glomerulonephritis were noted. Immunofluorescence analysis showed 2+ C3 and 1+ IgA granular patterns at the mesangium. Staining was negative for IgG, IgM, C1q, kappa, lambda, and fibrinogen. Right kidney biopsy was consistent with IgADIRGN. Whole-abdominal computed tomography (CT) was performed to seek the possible source of infection, which revealed an enlarged left kidney, staghorn stone obstructing dilated calyces, and gas collection within the collecting system (Figure 1A). The right kidney was normal in size. Enhanced CT of the left kidney revealed EPN class 2 according to the Huang and Tseng Classification (1). The patient was treated with intravenous ceftriaxone (2 g once daily for 7 days), and percutaneous catheter drainage was attempted to relieve obstruction in the urinary tract, but it failed due to unsuitable anatomy. The patient denied double J stenting placement because she felt that her symptoms improved after antibiotic treatment. After 7 days of intravenous ceftriaxone administration, she was treated as an outpatient, and the antibiotic was changed to oral ciprofloxacin, which was prescribed for 2 months. Her condition improved gradually; her appetite had improved, and she had gained 8 kg at the 4-month follow-up. However, her proteinuria persisted. After discussion between the attending physicians (including nephrologist and urologist) and patient, left nephrectomy was performed at 6 months after the first admission without any complications.

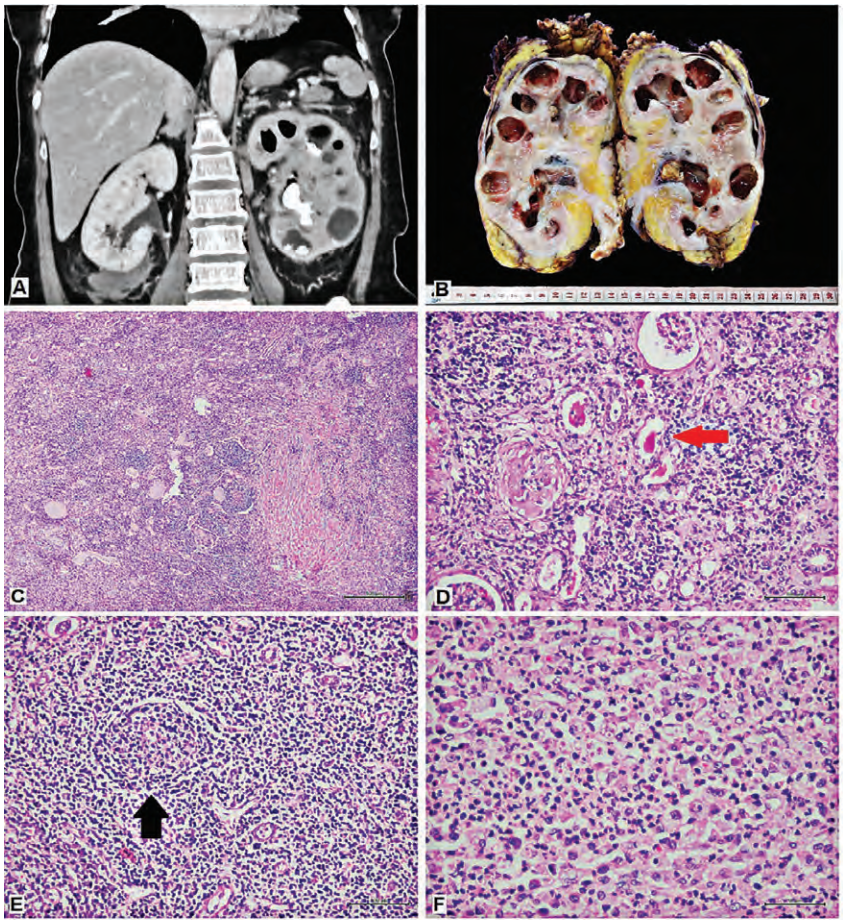

Figure 1. Coronal image of computed tomography, gross photograph of the kidney, and pathological finding. (A) Computed tomography of the abdomen shows an enlarged left kidney, multiple staghorn stones obstructing dilated calyces, and gas collection within the collecting system and renal parenchyma corresponding to class 2 (Huang and Tseng Classification) features. (B) Gross pathological analysis of the left kidney shows staghorn calculi, dilated calyces, and cystic cavities. (C) Microscopic examination reveals intense diffuse lymphoplasmacytic infiltration with interstitial fibrosis (hematoxylin and eosin staining, bar = 500 μm). (D & E) Tubular atrophy filled with colloid casts (red arrow) and germinal center (black arrow) are present (bar = 100 μm). (F) Focal abscessation and histiocytic infiltration are seen (bar = 50 μm).

Her 24-hour protein level reduced significantly 2 weeks following nephrectomy. Table 1 summarizes the patient's laboratory results during nephrectomy

and at the 2-week follow-up after nephrectomy. Light microscopy of the left kidney showed diffuse lymphoplasmacytic infiltration, interstitial fibrosis with focal abscessation, and histiocytic infiltration (Figure 1).

Discussion

We describe the case of a woman with EPN complicated by IgADIRGN who initially presented with unintentional weight loss and subnephrotic range proteinuria without fever. EPN and acute pyelonephritis have similar presenting symptoms; thus, making the diagnosis challenging. EPN symptoms rarely present without fever for >10 days before diagnosis (1). The unusual presenting symptoms complicated the diagnosis in our patient. Furthermore, our case highlights that CT provides greater accuracy in the diagnosis of EPN, as compared to ultrasonography. Renal ultrasound prior to renal biopsy showed nothing more than calyceal lithiasis with no signs of complication. Air-related lesion can be missed on ultrasonography in unsuspected cases.

The differential diagnoses were IgADIRGN and IgA nephropathy; however, a diagnosis of IgA nephropathy can only be established if immunofluorescence staining on kidney biopsy demonstrates dominant or co-dominant deposition of IgA nephropathy. Herein, kidney biopsy with immunofluorescence staining revealed a predominance of C3. The old-age onset, concurrent infection, and kidney pathology made a diagnosis of IgADIRGN more likely than one of IgA nephropathy; thus, no immunosuppressive agent or other treatments, apart from antibiotics, were prescribed. A potential cause of persistent post-renal proteinuria was EPN (same infection) or lithiasis that was resolved with nephrectomy. However, post-renal proteinuria is generally insignificant. Therefore, infection or lithiasis could not adequately explain the post-renal proteinuria in this case (2.1 g/day). After the diagnosis of IgADIRGN, infection as the cause of the patient's presenting symptoms was carefully investigated. IgADIRGN is a form of infection-related glomerulonephritis (6). It typically occurs in diabetic patients with staphylococcal infections (6) and can occur in nondiabetic patients with other pathogens (7). IgADIRN is being increasingly recognized and can present with multiple infection sites. It is imperative for caring physicians to be familiar with this entity.

Our patient had EPN complicated by IgADIRGN, which, to the best of our knowledge, has not been previously reported. In a retrospective study by John et al., urinary tract infection was the leading cause of parainfectious glomerulonephritis; however, they did not investigate infections of the upper or lower urinary tract or the severity of infection (8).

Limitation of Case Study

Study limitations include the lack of electron microscopy and no definitive diagnosis of IgADIRGN. The differential diagnoses of the right kidney pathology included IgADIRGN, IgA nephropathy, and C3 glomerulonephritis. The patient's concurrent infection, older age, and stronger staining for C3 than for IgA indicated that IgADIRGN was a more likely diagnosis (9). However, it is not always possible to distinguish these entities from each other even with the use of electron microscopy. Sometimes, it is not important to distinguish between secondary IgA nephropathy and IgADIRGN because both responded well to the treatment for the primary infection as in our case.

Conclusion

This case report described an unusual presentation of EPN, with a prolonged course of illness without fever. Moreover, IgADIRGN is an atypical complication of EPN that, to the best of our knowledge, has not been previously reported. Our report highlights the critical role of kidney biopsy in achieving accurate diagnosis and diverse clinical presentations in medicine.

What Is Already Known on This Topic:

Emphysematous pyelonephritis is a rare, life-threatening infection of the renal parenchyma that is associated with gas formation. Patients with

emphysematous pyelonephritis typically present with fever, flank pain, or septic shock in severe cases. A prolonged course of illness without fever prior to diagnosis is rare.

What This Case Adds:

This is believed to be the first reported case of emphysematous pyelonephritis presenting with a prolonged course of illness without fever and complicated by immunoglobulin A-dominant infection-related glomerulonephritis. This case study highlights the critical role of kidney biopsy in the diagnosis and the diverse clinical manifestations in clinical medicine.

Authors' Contributions: Conception and design: CC, PW, AC; Acquisition, analysis and interpretation of data: PW and PC; Drafting the article: KC; Revising it critically for important intellectual content: AC; Approved final version of the manuscript: KC, CC, and AC.

Conflict of Interest: The authors declare that they have no conflict of interest.

Consent to Publish Statement: Written informed consent was obtained from the patient for publication of this case report and all accompanying images.

Data Availability Statement: All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1. Huang JJ, Tseng CC. Emphysematous pyelonephritis: clinicoradiological classification, management, prognosis, and pathogenesis. Arch Intern Med. 2000;160(6):797-805. doi: 10.1001/archinte.160.6.797.
2. Eswarappa M, Suryadevara S, John MM, Kumar M, Reddy SB, Suhail M. Emphysematous Pyelonephritis Case Series From South India. Kidney Int Rep. 2017;3(4):950-5. doi: 10.1016/j.ekir.2017.12.003.
3. Aboumarzouk OM, Hughes O, Narahari K, Coulthard R, Kynaston H, Chlosta P, et al. Emphysematous pyelonephritis: Time for a management plan with an evidence-based approach. Arab J Urol. 2014;12(2):106-15. doi: 10.1016/j.aju.2013.09.005.
4. Michaeli J, Mogle P, Perlberg S, Heiman S, Caine M. Emphysematous pyelonephritis. J Urol. 1984;131(2):203-8. doi: 10.1016/s0022-5347(17)50309-2.
5. Chen MT, Huang CN, Chou YH, Huang CH, Chiang CP, Liu GC. Percutaneous drainage in the treatment of emphysematous pyelonephritis: 10-year experience. J Urol. 1997;157(5):1569-73.
6. Nasr SH, Fidler ME, Valeri AM, Cornell LD, Sethi S, Zoller A, et al. Postinfectious glomerulonephritis in the elderly. J Am Soc Nephrol. 2011;22(1):187-95. doi: 10.1681/ASN.2010060611.
7. Wen YK, Chen ML. IgA-dominant postinfectious glomerulonephritis: not peculiar to staphylococcal infection and diabetic patients. Ren Fail. 2011;33(5):480-5.
8. John EE, Thomas A, Eapen JJ, Yusuf S, Roy S, Valson AT, et al. Latency, Anti-Bacterial Resistance Pattern, and Bacterial Infection-Related Glomerulonephritis. Clin J Am Soc Nephrol. 2021;16(8):1210-20. doi: 10.2215/CJN.18631120.
9. Nasr SH, Radhakrishnan J, D'Agati VD. Bacterial infection-related glomerulonephritis in adults. Kidney Int. 2013;83(5):792-803. doi: 10.1038/ki.2012.407.

Images in Clinical Medicine
Acta Medica Academica 2022;51(1):64-65
DOI: 10.5644/ama2006-124.372

Large Bowel Obstruction Secondary to Urinary Retention

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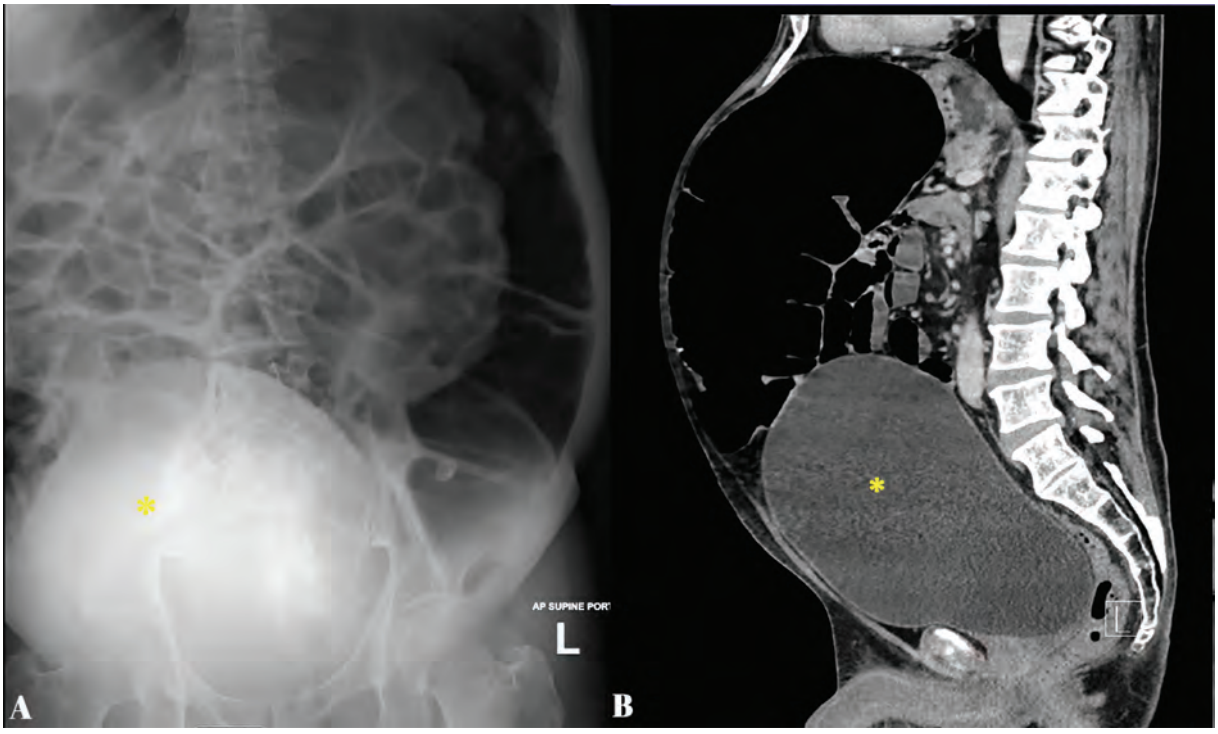
Received: 12 January 2022; **Accepted:** 17 February 2022

Key Words: Bowel Obstruction ▪ Urinary Retention ▪ CT Scan.



A 54-year-old man with past medical history of schizophrenia maintained on olanzapine and trihexyphenidyl presented with abdominal pain and distension, constipation and difficulty urinating. In physical exam abdominal distension with hyperactive bowel sounds were noted. Abdominal X-ray followed by CT scan with contrast showed gaseous distension throughout the colon with a transition point in proximal sigmoid colon in favor of large bowel obstruction and a remarkably distended bladder (Panel A and B). Bladder catheterization resulted in 3700 mL of urine. Nasogastric tube inserted and patient kept nil-per-os. Patient's abdominal discomfort improved

after catheterization. He started having bowel movements and tolerated diet over the next couple days. He recovered without need for surgical intervention and was discharged with stool softener and tamsulosin. Severe bladder distension is a rare cause of bowel obstruction (1). Bladder is a less mobile organ with lower intrapelvic fixation and its distention tends to pinch the rectosigmoid colon at the prominence of sacral promontory, where diameter of pelvis is narrow (2). In this case anticholinergic medication and prostate enlargement were contributing to urinary retention.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Papes D, Altarac S, Arslani N, Rajkovic Z. Urinary retention presenting as complete bowel obstruction. Can Urol Assoc J. 2013;7(9-10):E637-9.
2. Fujisaki T, Fujita Y, Mizuta H, Niina N, Miyazaki N, Tashiro A, et al. Large bowel obstruction caused by urinary retention from benign prostate hyperplasia. Radiol Case Rep. 2019;14(2):213-6.

Images in Clinical Medicine
Acta Medica Academica 2022;51(1):66-67
DOI: 10.5644/ama2006-124.373

A Case of Neglected Frontal Sinusitis Led to Frontal Sinus Empyema with Ocular Complications

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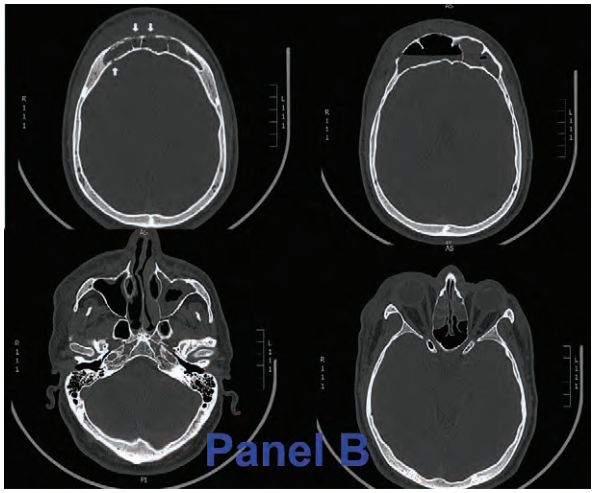
Received: 18 March 2022; **Accepted:** 25 April 2022

Key Words: Frontal Sinusitis ▪ Sinus Empyema ▪ Complications.



A 33-year-old male patient presented to the emergency department of our hospital with oedema and swelling over his forehead and upper right eyelid (Panel A). He complained of itching-eyes and a rash which had appeared 20 days earlier, and the swelling had appeared gradually over the next few days. He also reported a severe headache and the inability to open his right eye due to the swelling. The eyelid could hardly be retracted at all. Antibiotic drops prescribed by his ophthalmologist failed to resolve the symptoms. On clinical examination his vital signs were normal. Laboratory workup comprised a full blood count showing leukocytosis with neutrophilia, without any comorbidities.

Only *S. aureus* was isolated. The C-reactive protein was 7.7 mg/dl. Ophthalmological examination revealed decreased ocular mobility of the right eye due to swelling, but vision was unaffected. The top left image from a computed tomography scan (Panel B) shows orbital cellulitis and frontal sinus empyema with osteolysis of the posterior wall, which raised the suspicion of an intracranial complication. At first, he was treated with intravenous antibiotics for 24 hours. Since his condition worsened with dizziness and swelling, he was taken to the operating theatre. Under general anesthesia, he underwent functional endoscopic sinus surgery, including a Draft 2b procedure on the right side. He was discharged on the 4th post-op day on intranasal steroids, with intranasal washes with normal saline and oral antibiotics. Complete resolution was noted after two months (Panel C).

Acute rhinosinusitis (ARS) can lead to a variety of complications if it is left untreated or misdiagnosed. It has been reported that almost all infections of the frontal and maxillary sinuses are rhinogenic (1). Doctors should be aware of the serious potential complications of ARS, which range from orbital to frontal lobe abscesses. Management consists of a combination of systemic antibiotics and surgical drainage, with excellent outcome (2).

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. de Régloix SB, Maurin O, Crambert A, Genestier L, Bonfort G, Pons Y. Complications of sinusitis [in French]. Presse Med. 2017;46(7-8 Pt 1):655-9. doi: 10.1016/j.lpm.2017.05.027.
2. Al Yaeesh I, AlOmairin A, Al Shakhs A, Almomen A, Almomen Z, AlBahr A, et al. The serious complications of frontal sinusitis, a case series and literature review. J Surg Case Rep. 2020;2020(12):rjaa474. doi: 10.1093/jscr/rjaa474.

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ISSN 1840-1848