Official Publication of the Turkish Society of Anatomy and Clinical Anatomy

Aim and Scope
Anatomy, an international journal of experimental and clinical anatomy, is a peer-reviewed journal published three times a year with an objective to publish manuscripts with high scientific quality from all areas of anatomy. The journal offers a forum for anatomical investigations involving gross, histologic, developmental, neurological, radiological and clinical anatomy, and anatomy teaching methods and techniques. The journal is open to original papers covering a link between gross anatomy and areas related with clinical anatomy such as experimental and functional anatomy, neuroanatomy, comparative anatomy, modern imaging techniques, molecular biology, cell biology, embryology, morphological studies of veterinary discipline, and teaching anatomy. The journal is currently indexing and abstracting in TUBITAK ULAKBIM Turkish Medical Index, Proquest, EBSCO Host, Index Copernicus and Google Scholar.

Publication Ethics
Anatomy is committed to upholding the highest standards of publication ethics and observes the principles of Journal’s Publication Ethics and Malpractice Statement, which is based on the recommendations and guidelines for journal editors developed by the Committee on Publication Ethics (COPE), Council of Science Editors (CSE), World Association of Medical Editors (WAME) and International Committee of Medical Journal Editors (ICMJE). For detailed information please visit the online version of the journal which is available at www.anatomy.org.tr

Authorship
All persons designated as authors should have participated sufficiently in the work to take public responsibility for the content of the manuscript. Authorship credit should be based on substantial contributions to (1) conception and design or analysis and interpretation of data, (2) drafting of the manuscript or revising it for important intellectual content and, (3) final approval of the version to be published. The editor may require the authors to justify assignment of authorship. In the case of collective authorship, the key persons responsible for the article should be identified and others contributing to the work should be recognized with proper acknowledgment.

Copyright
Copyright © 2017, by the Turkish Society of Anatomy and Clinical Anatomy, TSACA. All rights reserved. No part of this publication may be reproduced, stored or transmitted in any form without permission in writing from the copyright holder beforehand, exceptionally for research purpose, criticism or review. The publisher and the Turkish Society of Anatomy and Clinical Anatomy assumes no liability for any material published in the journal. All statements are the responsibility of the authors. Although all advertising material is expected to conform ethical standards, inclusion in this publication does not constitute a guarantee or endorsement of the quality or value of such product or of the claims made of it by its manufacturer. Permission requests should be addressed to the publisher.

Publication Information
Anatomy (ISSN 1307-8798; eISSN 1308-6459) is published by Deomed Publishing, Istanbul, for the Turkish Society of Anatomy and Clinical Anatomy, TSACA. Due the Press Law of Turkish Republic dated as June 26, 2004 and numbered as 5187, this publication is classified as a periodical in English language.

Ownership
On behalf of the Turkish Society of Anatomy and Clinical Anatomy, Ahmet Kağan Karabulut, MD, PhD, Ankara

Responsibility Managing Editor
Nihat Apaydın, MD, PhD, Ankara

Administrative Office
Güven Mah. Güvenlik Cad. Onlar Av. 129/2 Aşağı Ayvano, Ankara
Phone: +90 312 447 55 52-55

Publisher
Deomed Publishing
Gür Sok. No:78 Karaköy, Istanbul, Turkey
Phone: +90 216 414 83 43 (Pbo) / Fax: +90 216 414 83 42
www.deomed.com / e-mail: medya@deomed.com

Submission of Manuscripts
Contributions should be submitted for publication under the following categories to:
Gülşin Sengül, MD
Editor-in-Chief, Anatomy
Department of Anatomy, Faculty of Medicine, Ege University, 35100, Bornova, İzmir, Turkey
Phone: 090 232 390 39 84
Fax: 0090 232 342 21 42
E-mail: gulsin.sengul@gmail.com; gulsin.sengul@ege.edu.tr

Categories of Articles
- Original Articles describe substantial original research that falls within the scope of the Journal.
- Teaching Anatomy section contains regular or all formats of papers which are relevant to comparing teaching models or to introducing novel techniques, including especially the own experiences of the authors.
- Reviews section highlights current development in relevant areas of anatomy. The reviews are generally invited, other prospective authors should consult with the Editor-in-Chief.
- Case Reports include new, noteworthy or unusual cases which could be of help for basic notions and clinical practice.
- Technical Note articles cover technical innovations and developments with a specific technique or procedure or a modification of an existing technique. They should be sectioned like an original research article but not exceed 2000 words.
- Viewpoint articles give opinions on controversial topics or future projections, some of these are invited.
- Historical View category presents overview articles about historical sections from all areas of anatomy.
- Terminology Zone category is a platform for the articles which discuss some terminological controversies or opinions.

The categories above are peer-reviewed. They should include abstract and keywords. There are also categories including Letters to the Editor, Book Reviews, Abstracts, Obituary, News and Announcements which do not require a peer review process.

For detailed instructions concerning the submission of manuscripts, please refer to the Instructions to Authors.

Subscription
Please send your order to Deomed Publishing, Gür Sok. No: 78/8 Kadıköy, Istanbul, Turkey. e-mail: medya@deomed.com

- Annual rates: Institutional 100 EUR, Individual 50 EUR (include postage and local VAT). Supplements are not included in the subscription rates.
- Membership of the Turkish Society of Anatomy and Clinical Anatomy, TSACA includes a reduced subscription rate to this journal.
- Change of address: Please send to the publisher at least six weeks in advance, including both old and new addresses.
- Cancellations: Subscription cancellations will not be accepted after the first issue has been mailed.

The online version of this journal is available at www.anatomy.org.tr

Advertising and Reprint Requests
Please direct to publisher. e-mail: medya@deomed.com

Printing and Binding
Birmat Press, Istanbul, Turkey. Phone: +90 212 629 05 59-60
Printed in Turkey on acid-free paper (December 2017).
Anatomy, an international journal of experimental and clinical anatomy, is the official publication of the Turkish Society of Anatomy and Clinical Anatomy, TSACA. It is a peer-reviewed journal that publishes scientific articles in English. For a manuscript to be published in the journal, it should not be published previously in another journal or as full text in congress books and should be found relevant by the editorial board. Also, manuscripts submitted to Anatomy must not be under consideration by any other journal. Relevant manuscripts undergo conventional peer review procedure (at least three reviewers). For the publication followed, author(s) should report the duration of any conflict of interest and transfer the copyright to the Turkish Society of Anatomy and Clinical Anatomy, TSACA.

In the Materials and Methods section of the manuscripts where experimental studies on humans are presented, a statement that informed consent was obtained from each volunteer or patient after explanation of the procedures should be included. This section also should contain a statement that the investigation conforms with the principles outlined in the appropriate version of 1964 Declaration of Helsinki. For studies involving animals, all work must have been conducted according to applicable national and international guidelines. Prior approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from the relevant author’s institutional or other appropriate ethics committee, and the institution name and approval must have been obtained for all protocols from

Anatomical terms used should comply with Terminologia Anatomica by FCAT (1998).

No publication cost is charged for the manuscripts but reprints and color printings are at authors’ cost.

Preparation of manuscripts During the preparation of the manuscripts, uniform requirements of the International Committee of Medical Journal Editors, a part of which is stated below, are valid (see ICMJE. Uniform requirements for manuscripts submitted to biomedical journals. Updated content is available at www.icmje.org). The manuscript should be typed double-spaced on one side of a 21 x 29.7 cm (A4) blank sheet of paper. At the top, bottom and right and left sides of the pages a space of 2.5 cm should be left and all the pages should be numbered except for the title page.

Manuscripts should not exceed 15 pages (except for the title page). They must be accompanied by a cover letter signed by corresponding author and the Conflicts of Interest Disclosure Statement and Copyright Transfer Form signed by all authors. The contents of the manuscript (original articles and articles for Teaching Anatomy category) should include: 1- Title Page, 2- Abstract and Keywords, 3- Introduction, 4- Materials and Methods, 5- Results, 6- Discussion (Conclusion and/or Acknowledgement if necessary), 7- References

Title page In all manuscripts the title of the manuscript should be written at the top and the full names and surnames and titles of the authors beneath. These should be followed with the affiliation of the author. Manuscripts with long titles are better accompanied underneath by a short version (maximum 80 characters) to be published as running head. In the title page the correspondence address and telephone, fax and e-mail should be written. At the bottom of this page, if present, funding sources supporting the work should be written with full names of all funding organizations and grant numbers. It should also be indicated in a separate line if the study has already been presented in a congress or likewise scientific meeting. Other information such as name and affiliation are not to be indicated in pages other than the title page.

Abstract Abstract should be written after the title in 100–250 words. In original articles and articles prepared in IMRAD format for Teaching Anatomy category the abstract should be structured under sections Objectives, Methods, Results and Conclusion. Following the abstract at least 3 keywords should be added in alphabetical order separated by semicolons.

References Authors should provide direct references to original research sources. References should be numbered consecutively in square brackets, according to the order in which they are first mentioned in the manuscript. They should follow the standard detailed in the NLM’s Citing Medicine, 2nd edition (Citing medicine: the NLM style guide for authors, editors, and publishers [Internet]. 2nd edition. Updated content is available at www.ncbi.nlm.nih.gov/books/NBK7256). The names of all contributing authors should be listed, and should be in the order they appear in the original reference. The author is responsible for the accuracy and completeness of references. When necessary, a copy of a referred article can be requested from the author. Journal names should be abbreviated as in Index Medicus. Examples of main reference types are shown below:

- **Journal articles:** Author’s name(s), article title, journal title (abbreviated), year of publication, volume number, inclusive pages
- **Books:** Author’s name(s), book title, place of publication, publisher, year of publication, total pages (entire book) or inclusive pages (contribution to a book or chapter in a book)
- **Entire book:**
  - Contribution to a book:
  - Chapter in a book:

Illustrations and tables Illustrations and tables should be numbered in different categories in the manuscript and Roman numbers should not be used in numbering. Legends of the illustrations and tables should be added to the end of the manuscript as a separate page. Attention should be paid to the dimensions of the photographs to be proportional with 10x15 cm. Some abbreviations out of standards can be used in related illustrations and tables. In this case, abbreviation used should be explained in the legend. Figures and tables published previously can only be used when necessary for a comparison and only by giving reference after obtaining permission from the author(s) or the publisher (copyright holder).

Control list
- Length of the manuscript (max. 15 pages)
- Manuscript format (double space; one space before punctuation marks except for apostrophes)
- Title page (author names and affiliations; running head; correspondence)
- Abstract (100–250 words)
- Keywords (at least three)
- References (referred to Index Medicus)
- Illustrations and tables (numbering; legends)
- Conflicts of Interest Disclosure Statement and Copyright Transfer Form
- Cover letter
Introduction

Mustard seeds are the tiniest part of the mustard plant which flourishes in the cold weather, moist soil and general temperate conditions. The plant grows as a shrub while the seeds are about two millimeters in diameter and used as spices in many countries. There are approximately 40 different varieties of mustard plants, but the three principal ones which also vary in color are *Brassica hirta* or *alba* (yellow-white), *Brassica nigra* (black) and *Brassica juncea* (brown). Mustard seeds, apart from being a culinary essential, have had medicinal applications dating back to the time of Hippocrates. It has in fact been once mentioned as the greatest herb ever. We explored the dose dependent effects of the crude aqueous extract of *Brassica nigra* (Black mustard seeds) on the prefrontal cortex of adult Wistar rats.

Methods: 20 adult female rats weighing an average of 180±20 g were used. They were split into 4 groups (n=5); Group A (received extract at 200 mg/kg body weight), Group B (received extract at 100 mg/kg body weight), Group C (received extract at 50 mg/kg body weight), and Group D (received distilled water *ad libitum*). All of the animals were subjected to the Y-maze spontaneous alternation test for neurobehavioural analyses following 28-day administration of the extracts. Animals were sacrificed 24 hours after taking the last day of administration.

Results: Our results showed that neurobehavioural analyses are significantly hampered in animals receiving 200 mg/kg extract in comparison to the control group. In treatment groups, increased dose of extract elevated the level of MDA, but reduced the level of SOD. LDH levels were also significantly increased in the 200 mg/kg treated group when comparing with the control. General microarchitecture in the prefrontal cortex of 200 mg/kg *Brassica nigra* treated group showed signs of karyolysis and pyknosis.

Conclusion: *Brassica nigra* (black mustard) is not innocuous and therefore it should be consumed in moderation.

Keywords: *Brassica nigra*; neurobehaviour; neurototoxicity; prefrontal cortex

Dose-dependent effect of black mustard seeds (*Brassica nigra*) extract on the prefrontal cortex of adult Wistar rats

Tolulope Timothy Arogundade¹², Bernard Ufuoma Eneibe¹², Oluwaseun Olaniyi Adigun¹, Foyeke Munirat Adigun², Ismail Temitayo Gbadamosi²

¹Department of Anatomy, Faculty of Basic Medical Sciences, Adeleke University, Ede, Nigeria
²Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria

Abstract

Objectives: Mustard seeds, apart from being a culinary essential, have had medicinal applications dating back to the time of Hippocrates. It has in fact been once mentioned as the greatest herb ever. We explored the dose dependent effects of the crude aqueous extract of *Brassica nigra* (Black mustard seeds) on the prefrontal cortex of adult Wistar rats.

Methods: 20 adult female rats weighing an average of 180±20 g were used. They were split into 4 groups (n=5); Group A (received extract at 200 mg/kg body weight), Group B (received extract at 100 mg/kg body weight), Group C (received extract at 50 mg/kg body weight), and Group D (received distilled water *ad libitum*). All of the animals were subjected to the Y-maze spontaneous alternation test for neurobehavioural analyses following 28-day administration of the extracts. Animals were sacrificed 24 hours after taking the last day of administration.

Results: Our results showed that neurobehavioural analyses are significantly hampered in animals receiving 200 mg/kg extract in comparison to the control group. In treatment groups, increased dose of extract elevated the level of MDA, but reduced the level of SOD. LDH levels were also significantly increased in the 200 mg/kg treated group when comparing with the control. General microarchitecture in the prefrontal cortex of 200 mg/kg *Brassica nigra* treated group showed signs of karyolysis and pyknosis.

Conclusion: *Brassica nigra* (black mustard) is not innocuous and therefore it should be consumed in moderation.

Keywords: *Brassica nigra*; neurobehaviour; neurototoxicity; prefrontal cortex

damage by assessing the level of superoxide dismutase of the PFC in Wistar rats. We examined the oxidative functions, oxidative parameters, and finally, the histology of Brassica nigra. We generally examined the dose-dependent effects of aqueous extracts on the cognitive and memory systems. The frontal cortex modifies those emotions to fit socially acceptable norms. Thus, we specifically examined the dose-dependent effects of aqueous *Brassica nigra* seed extracts on the cognitive and memory functions, oxidative parameters, and, finally, the histology of the PFC in Wistar rats. We examined the oxidative damage by assessing the level of superoxide dismutase (SOD), malondialdehyde (MDA) and lactate dehydrogenase (LDH) enzymes.

### Materials and Methods

All protocols and treatment procedures were done according to the Institutional Animal Care and Use Committee (IACUC) guidelines and as approved by the Faculty of Basic Medical Sciences Ethics Review Committee, University of Ilorin, Nigeria. Twenty (20) adult female Wistar rats with an average weight of 180±20 g were purchased from a private animal holding in Ilorin and used for the research. These rats were kept in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. The rats had liberal access to rat chow and water.

Animals were randomly assigned into 4 groups (A–D), each consisting of 5 rats. The groups were treated as follows: Group A received 200 mg/kg of extract daily for 28 days; Group B received 100 mg/kg for 28 days; Group C received 50 mg/kg for 28 days, and Group D received distilled water for 28 days. The rats were weighed twice a week (72 hours of intervals), beginning from the first day of administration using a digital weighing balance.

Mustard seeds were obtained from a local market in Ilorin. It was certified true by the department of Plant Biology at the University of Ilorin. The extract was prepared according to the method described by Inyang et al. The mustard seeds (*Brassica nigra*) were pulverized into powder with an electric blender. Three hundred grams (300 g) of the ground seeds was soaked in 1000 ml of distilled water; stirred and left for 72 hours in a refrigerator at 4°C. The mixture was sieved and filtered with Whatman No.1 filter paper. The resultant filtrate was dried in a water bath at 40°C for 96 hours to get the concentrate which was then diluted to stock. The extract was administered via the oral route. Proper volume was ensured through the use of a calibrated syringe fitted with an oral cannula.

Y-maze spontaneous alternation test was used to assess working and cognitive memory in rodents. Alternation behavior is based on the natural tendency in rodents to explore the maze systematically entering each arm in turn. Alternation behavior is then defined as consecutive entries into each of the three arms without repetition. This was examined using a Y-maze composed of three equally spaced arms (120°, 41 cm long and 15 cm high). The floor of each arm is made of plywood and is 5 cm wide. Briefly, each rat was placed in one of the arm compartments and was allowed to move freely until its tail completely enters another arm. The sequence of arm entries was manually recorded with a video camera, the arms being labeled A, B, or C. An alternation is defined as entry into all three arms consecutively, for instance if the animal makes the following arm entries: A, C, B, C, A, B, C, A, C, A, B, C, A, in this example, the animal made 13 arm entries 8 of which are correct alternations. The number of maximum spontaneous alternations is then the total number of arms entered minus two, and the percentage alternation is calculated as [(actual alternations/maximum alternations) × 100]. For each animal in a group the Y-maze testing was carried out for 5 minutes, three trials and recorded for later analyses. The videos were analyzed by a neutral observer to eliminate bias.

After completion of treatments, rats were euthanized using 20 mg/kg of ketamine (i.p.) for histological analysis. Transcardiac perfusion was done by exposing the left ventricle and injecting 50 ml 0.1 M PBS (pH 7.4) followed by 400 ml 4% paraformaldehyde (PFA) while the rat was suspended in an inverted position (gravity). Dissected brains were then rinsed in 0.25 M sucrose 3 times for 5 minutes each and then post fixed in 4% PFA for 24 hours before being stored in 30% sucrose at 4°C until further processing. Rats for enzymatic assays were sacrificed by separating the head from the trunk, to avoid the interference of ketamine with biochemical redox; brains were then excised, rinsed in 0.25 M sucrose 3 times for 5 minutes each and placed in 30% sucrose in which they were stored at 4°C. Coronal sections of PFC and hippocampus were obtained according to stereotoxic coordinates (+4 mm) from each brain. Histological staining was carried out in paraffin wax embedded sections which were stained with Haematoxylin and Eosin (H&E) using the methods described by Fischer et al. Histochemical demonstration of Nissl substances was done with slight modification to the method published by Kádár et al.
Determination of SOD, LDH and MDA activities was carried out on whole brains of treated rats using spectrophotometric technique. Each of the assay kits were procured from Bio Legend Inc., San Diego, CA, USA. Whole brain (in sucrose at 4°C) from rats across groups were weighed and pulverized in 0.25 M sucrose (Sigma) with the aid of an automated homogenizer at 4°C. Lysates from the brain were centrifuged for 10 minutes in a microcentrifuge at 12,000 rpm to obtain the supernatant containing organelle fragments and synaptosomes. The supernatants were aspirated into plain labeled glass cuvette placed in ice. SOD, LDH and MDA activities were assayed according to the manufacturer’s instruction in the assay kit pack.

PFC sections on glass slides were captured using Olympus binocular research microscope (Olympus, Morristown, NJ, USA) which was connected to a 5.0 MP Amscope Camera (Amscope Inc, Irvine, CA, USA).

All quantitative data were analyzed using GraphPad Prism v.6 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS (Statistical Package for the Social Sciences, version 20.0; IBM, Chicago, IL, USA) software. Body weight, brain weight, spontaneous alternation, lactate dehydrogenase (LDH), malondialdehyde (MDA), and superoxide dismutase (SOD) outcomes were plotted in ANOVA followed with Tukey’s multiple comparisons test. Significance was set at p<0.05 and p<0.01. The results were represented in bar charts with error bars to show the mean and standard error of mean (mean±SEM) respectively.

**Results**

**Body and brain weights of experimental animals**

As described in the methods section, the body weight changes of the experimental rats was monitored and evaluated at a 72-hour interval following the beginning of administration and differences were examined by the analysis of variance (ANOVA). At the end of the first week of administration, there was a significant (p<0.05) increase in the body weight of animals in Group A (200 mg/kg) and Group B (100 mg/kg) relative to the control (Figure 1). Body weight gain of the low dosage group (50 mg/kg) was not significantly different than those of control group. During the following weeks of administration, differences in the body weights of animals among the experimental groups were abolished. The mean brain weight of treated animals was not significantly different than control groups (Figure 2).

**Y-maze test results display dose dependence**

The correct percentage alternation in Group A, which received the extract at the dosage of 200 mg/kg body weight revealed a significant reduction (p<0.05) in comparison to the control (Figure 3). However, rats received
the extract at 50 mg/kg body weight dosages seemed to perform usually better in the alternation test in compared to those of the control. However, this observation was statistically insignificant. Group B that is received the extract at the dose of 100 mg/kg body weight was displayed relatively similar performance to the control group (Group D).

**Alterations in the oxidative damage markers**

MDA profiles displayed an augmentation in the treatment groups in parallel to the increasing dose of *Brassica nigra* extract, but this increase was not significantly different than those of the control group (Figure 4).

**High dose *Brassica nigra* treatment associates with LDH overexpression**

LDH is expressed extensively in body tissues during tissue damage. LDH level was significantly increased (p<0.01) in animals who received *Brassica nigra* extract at 200 mg/kg body weight dose) when compared with the control and low dose treatment groups (Figure 5). In animals receiving the a mild dose of (100 mg/kg body weight) extract there was also a slight increase, but it was not enough to be statistically significant in comparison to the control group. These results showed some consistence with earlier findings of this study, suggesting the involvement of *Basilica nigra* in cellular damage. The levels of LDH enzyme in animals received the low dose (50 mg/kg body weight) of the extract was comparable to the control animals (Figure 5).

**SOD profiles decreases following *Brassica nigra* administration**

Differential expression of SOD was assessed in the brain tissues of treated rats. Results from spectrophotometric assay showed that SOD levels in the low (50 mg/kg body weight) and medium (100 mg/kg body weight) dose treatment groups increased in comparison to the control, but this rise was not significantly different. SOD level in the high dose group (200 mg/kg body weight), on the other hand was significantly lower than those of other groups (Figure 6).

**Histological changes in the prefrontal cortical areas**

The cellular anatomy of the prefrontal cortex in the control, low (50 mg/kg) and medium (100 mg/kg) doses of treatment with *Brassica nigra* seeds extract groups were dominated by large neurons characterized by the distinct pyramidal shape of their soma (Figure 7). However, *Brassica nigra* seeds extract at the 200 mg/kg body weight...
Doses induced microarchitectural damages in the prefrontal cortex of animals by displaying properties of early stage apoptosis characterized by karyolysis and pyknosis. On histologic examination with H&E staining, apoptosis involves single cells or small clusters of cells as shown by the dotted circles around the degenerating neurons in the 200 mg/kg treated group and the apoptotic cell appears as a round or oval mass, with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments. Furthermore, the cryptic changes seen in the soma of pyramidal neurons of the 200 mg/kg adult treated group also suggests apoptotic mode of neuronal cell death, in which cell shrinkage made the cells smaller in size, with dense cytoplasm and the organelles more tightly packed. Cresyl fast violet staining for Nissl substance, on the other hand, revealed a reduction in the staining intensity across all Basilica nigra treated groups in comparison to the control group (Figure 8).

Figure 7. Histological analysis of PFC morphology revealed by H&E staining (a–d: ×100, e–h: ×400). (a) 200 mg/kg, (b) 100 mg/kg, and (c) 50 mg/kg Basilica nigra, (d) control. The molecular layer (I), external granular layer (II), and external pyramidal layer (III) are shown across study groups. PFC cytoarchitecture of the control group are in normal array and succinctly layered, while PFC neuropil especially in the 200 mg/kg Basilica nigra group appeared charred, characterized by several fragmentations with a number of vacuolated cells (thick black arrow), dark pyknotic neurons (thin black arrows) and charred neuropil (star). [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]

Figure 8. Histochemical demonstration of PFC morphology in treated rats shown in cresyl fast violet for Nissl substance (a–d: ×100, e–h: ×400). (a) 200 mg/kg, (b) 100 mg/kg, and (c) 50 mg/kg Basilica nigra, (d) control. Similar to histological observations, control group appear morphologically normal and densely populated by Nissl proteins (cytoplasmic basophilic masses). Pyramidal neurons with notable processes are observable within the neuropil of the control group. Intensity of staining appears to be low across Basilica nigra treated groups. Cells with condensed irregular nuclei and peripheral vacuolation (thick black arrow), cells with low number of Nissl granules (thin arrows). [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]
Discussion

Mustard seeds have been used for medicinal applications dating back to the time of Hippocrates. It has been largely greeted for its healing properties and indicated as a potential cure-all. As early as 1699, it was claimed that mustard seed could strengthen the memory, expel heaviness, and revive the spirits. Mustard seed was recommended in 1653 for toothaches, joint pain, skin problems, and stomach aches. This present study investigated the effects of *Brassica nigra* (black mustard) on the prefrontal cortex of adult Wistar rats at different doses (200 mg/kg, 100 mg/kg, and 50 mg/kg).

At the end of the first week of administration, there was a significant increase in the body weight gains of animals treated with the doses of 200 mg/kg and 100 mg/kg seeds extracts in comparison to the control. The following weeks of administration, we saw subtle increases in the body weight of each experimental group. Mustard seeds have been attributed to the appetite stimulating properties and this might be the probable reason for the increment in the body weight of animals in a dose-dependent manner. Appetite is the psychological desire to eat, and it is controlled by the lateral nucleus of the hypothalamus. It is sometimes triggered by hunger, but many times it is due to cravings, habits, the availability of food, boredom or other social and emotional factors. It is possible that the crude aqueous *Brassica nigra* seeds extract might stimulate the hypothalamic lateral nuclei and the appetite of animals to an extent that the animals eat not really out of hunger, but from the mere availability of food, and this could be the reason for the higher weight gain across treatment groups when compared with the control group. Kumar et al. similarly reported an increased weight gain in animals treated with *Brassica nigra* at 500 mg/kg and 1000 mg/kg when compared with the control in their experiment assessing the antihyperglycemic and antioxidant activity of *Brassica nigra* oil in streptozotocin-induced diabetic rats. They, however, used the oil extract from the *Brassica nigra* seeds.

Following sacrifice, the average brain weights of animals across groups were measured and significant difference was observed in the mean brain weights of animals treated with *Brassica nigra* seeds extract.

Evaluation of the behavioral outcomes provides important cues in assessing the effects of the treatment. As a correlative test for cellular, molecular and neuropathological changes within the PFC in this study, we assessed alternation behavior in treated rats. Y-maze spontaneous alternation test is used for the behavioral assessment, based on the natural tendency of rodents in exploring the maze systematically and entering the each arm in turn. The result of this test revealed a significant reduction in the correct percentage alternation in animals receiving the extract at 200 mg/kg body weight doses when compared with the control group. Animals that received the extract at 50 mg/kg doses had a slightly higher, but not statistically significant, correct percentage alternation score in comparison to the control group. Group B that received the extract at 100 mg/kg doses was relatively at par with the control group. These results showed that at a relatively low to moderate doses (50 mg/kg and 100 mg/kg), *Brassica nigra* was somewhat beneficial to cortico-hippocampal dependent cognition and working memory, but at a higher dose (200 mg/kg), it impacts negatively. These results suggest that *Brassica nigra* might act as a “double-edged sword”. The possibility that alteration of normal mitochondrial redox and glucose bioenergetics dysfunction initiated by high doses of *Brassica nigra* is responsible for the impaired performance of treated rats in the Y-maze recorded in this study.

The biochemical indices monitored in the brain are useful “markers” for assessment of tissue integrity. The measurement of activities of various enzymes in the tissues and body fluids plays significant role in disease investigation and diagnosis. Tissue enzymes can also indicate cellular damage caused by chemical compounds in the extract long before structural damages that can be picked up by conventional histological techniques. In the absence of oxygen, the brain derives its required ATP through anaerobic glycolysis and LDH is needed for the conversion of lactate to pyruvate. LDH is expressed extensively in body tissues. It is released during tissue damage. LDH levels in the prefrontal cortex was significantly increased in animals that received *Brassica nigra* extract at 200 mg/kg doses) when compared with the control. This is indicative for the tissue damage and might be the reason for the poor performance in the Y maze spontaneous alternation test observed in this group (200 mg/kg). Its level in animals that received the extract at a milder dose (100 mg/kg) also showed an increase, but it was not enough to be statistically significant in comparison to the control group. It was however observed that the levels of LDH enzyme in Group C animals, which received the low dose (50 mg/kg) of the extract, were relatively at par with the control animals. This increase in the activity of LDH indicates an increase in carbohydrate metabolism for energy production via the glycolytic pathway. Malondialdehyde (MDA) is a marker for lipid peroxidation that indicates the oxidative degradation of lipids. It is the process in which free radicals “steal” electrons from the lipids in cell membranes, caused by cellular damage. MDA levels increased in the treated groups with increasing dose of *Brassica nigra* extract (50 mg/kg, 100 mg/kg, and 200 mg/kg), when compared with the control group.
although it did not reach to the significance levels. This observation, is contradictory to the results of Kiasalari et al. who showed a decrease in the level of MDA in brain tissues.

Since superoxide is one of the main reactive oxygen species in the cell. SOD serves a key antioxidant role. SOD decreases oxidative stress and generation of the reactive oxygen species. Therefore, inhibition of the endothelial activation indicates modulation of factors that govern adhesion molecule expression and leukocyte-endothelium interactions. While SOD levels display enhancement in animals treated with the low (50 mg/kg body weight) and medium (100 mg/kg body weight) doses of the extract in comparison to the control, it showed a significant reduction in animals receiving the high (200 mg/kg) dose. The former (50 mg/kg and 100 mg/kg) gives truth to the claim that *Brassica nigra* has antioxidative properties. This result however, further suggests that *Brassica nigra* exhibits some degree of paradoxical functional facilitation i.e. it is detrimental at a high dose, but is potentially beneficial at a low to medium dose. Kiasalari et al. observed that SOD level increased in animals treated with 150 mg/kg body weight of hydro-alcoholic *Brassica nigra* seeds extract in their experiment on evaluating the antiepileptic and antioxidant effect of *Brassica nigra* on pentylentetrazol-induced kindling mice.

Our observations on the neuronal morphology in the prefrontal cortical region suggest appropriate and normal functionality in these animals since, from the functional point of view, these neurons are the most important cells of the brain. *Brassica nigra* seeds extract at 200 mg/kg body weight induced microarchitectural damages in the adult group presented with properties of early stage apoptosis as apoptotic cells are characterized by karyolysis and pyknosis. On histologic examinations with classical staining, degenerating neurons were recognized by their characteristics appearances in animals treated with 200 mg/kg doses of the extract. Similar neuronal apoptotic bodies have been previously described consisting of cytoplasm with tightly packed organelles, with or without a nuclear fragment. Cresyl fast violet staining for Nissl substance revealed a reduction in the staining intensity across all *Basilica nigra* treated groups when compared with the control. This is suggestive of decreased synthesis of proteins by the rough endoplasmic reticulum in the cells.

**Conclusion**

*Brassica nigra*‘s appetite stimulating property was observed in this study. The present research also indicated that *Brassica nigra* is not totally innocuous. At the high (200 mg/kg) doses, the crude aqueous extract of the seeds have insulting effects on the prefrontal cortex microarchitecture of adult albino Wistar rats, as well as the expression and activities of certain enzymes, most probably due to the generation of free radicals and oxidative stress.

**References**


Introduction

Stressful experiences during pregnancy might lead to diverse consequences including neuroendocrine abnormalities, metabolic disorders, behavioral and cognitive problems in the offspring.\[1,2\] Prenatal stress (PS) exposure disturbs the brain development of pups by altering expression of stress hormones, neurotransmitters and their receptors,\[3,4\] activity of several ion channels\[5\] and synaptic formation.\[6\] Numerous studies have shown the effects of PS on the limbic structures, such as the hippocampus and the prefrontal cortex,\[7,8\] whereas relatively little is known about effects on the other limbic structures. The nucleus accumbens (NAc) is the center of reward mood and motivation, but it has recently been implicated in the pathophysiology of depression especially via dopaminergic and glutamatergic alterations.\[9–12\] As a major component of the ventral striatum, NAc extends dorsolaterally into the putamen and dorsomedially into the caudate nucleus.\[13\] The NAc contains a large number of γ-aminobutyric acid (GABA)-containing medium spiny projecting neurons (%95) and only a small populations of GABAergic and cholinergic interneurons.\[14\] The activity of medium spiny neurons is regulated by glutamatergic afferents coming from the prefrontal cortex, hippocampus and amygdala, dopaminergic

Single dose ketamine injection affects activation of cells in the nucleus accumbens of prenatally stressed rats

Elif Polat Çorumlu\(^1\), Osman Özcan Aydın\(^1\), Emel Ulupınar\(^1,2\)

\(^1\)Interdisciplinary Neuroscience Department, Osmangazi University, Eskisehir, Turkey
\(^2\)Department of Anatomy, Faculty of Medicine, Osmangazi University, Eskisehir, Turkey

Abstract

Objectives: The nucleus accumbens (NAc) has recently been implicated in the pathophysiology of depression. In animals displaying depressive-like behavior following chronic stress exposure, glutamatergic transmission increases in the NAc. NMDA receptor antagonist ketamine act as an antidepressant, especially in treatment-resistant cases. In this study, we aimed to investigate the effects of single-dose ketamine application in the activation of cells in the NAc of prenatally stressed rats.

Methods: Sprague-Dawley dams were exposed to immobilization stress during the last week of pregnancy for 3 hours. Male offspring were divided into four groups at postnatal day 40. Prenatal stress and control groups received either a single dose of ketamine (10 mg/kg, i.p.) or same doses of saline injections. Immediate gene activation was stimulated by forced swim for 6 minutes and assessed by c-Fos immunohistochemistry. The total number of activated cells in the core and shell subregions was estimated by optical fractionator method.

Results: The total number of activated cells in the shell subregion significantly decreased in prenatally stressed rats. Ketamine administration reversed their activation level similar to those of control group. Although activation of cells did not change in the core region following prenatal stress, ketamine treatment enhanced the activation of cells in this region both control and prenatally stressed animals.

Conclusion: These results suggest that prenatal stress influences the activation of NAc in a subdivision specific manner, but ketamine treatment could act on both core and shell regions by affecting glutamatergic limbic information that flows from shell to core subregion of the NAc.

Keywords: c-Fos; ketamine; nucleus accumbens; prenatal stress; stereology

Anatomy 2017;11(3):115–120 ©2017 Turkish Society of Anatomy and Clinical Anatomy (TSACA)
afferents from the ventral tegmental area, as well as serotonergic and noradrenergic afferents from the raphe nucleus, and the locus ceruleus, respectively. Thus, the NAc plays important roles in emotional control.

The NAc is divided into a central core surrounded by an outer shell area on its medial, ventral and ventrolateral sides. These two anatomically and functionally distinct subregions have diverse organizational patterns. While the core region projects primarily to a restricted part of the globus pallidus and the substantia nigra; the shell region projects not only to the subcommisural part of the ventral pallidum and the ventral tegmental area (VTA), but also projects to widespread areas in the hypothalamus and extended amygdala. Shell area contains substances such as calcretinin, substance P, dopamine, and serotonin, while the core area contains calbindin, encephalin and GABA A receptors. Furthermore, Fos-like immunoreactivity is higher in the shell region of the NAc.

In animals displaying depression-like behavior following chronic stress exposure, glutamatergic transmission increases in the NAc. Intriguingly, susceptibility to depression is mediated by glutamatergic transmission from the ventral hippocampal afferents to NAc, but not from the medial prefrontal cortex or basolateral amygdala. Because in vivo optogenetic manipulations of ventral hippocampus-NAc synaptic transmission cause depressive-like behaviors, while acute enhancement of glutamatergic input from either medial prefrontal cortex or basolateral amygdala attenuates transmission and promotes resilience. Recently, inhibition of glutamate release in subregions of the hippocampus and prefrontal cortex has been shown as a promising target in the treatment of patients who do not respond to classical antidepressants. It has been demonstrated that NMDA receptor antagonist ketamine shows such an antidepressant effect by rapidly stimulating the hippocampus and prefrontal cortex has been shown as a specific manner.

Materials and Methods
Healthy Sprague-Dawley rats obtained from the breeding colony at the Eskisehir Osmangazi University Animal Care Facility and maintained under constant temperature (21°C) and light (12:12 h light/dark cycle) conditions. After overnight mating, pregnancy was confirmed by the sperm positivity in the vaginal smear. Pregnant rats were housed individually in transparent cages by giving free access to food and tap water. Experimental procedures were performed in accordance with protocols approved by the Institutional Animal Usage Committee (Protocol #201/1). Dams (n=10) were immobilized in close-fitting wire mesh cylinders daily for 3 hours, between E14 and E21. At postnatal day 40, male offspring from control and prenatally stressed groups (n=20) received serum physiologic (CTRL+SF and PS+SF) or a single dose (10 mg/kg, i.p.) of ketamine injection (CTRL+ketamine and PS+ketamine) Animals were sacrificed two days after ketamine or SF injection. Forced swim procedure was used to stimulate neuronal activity for evaluation of the immediate gene expression. Animals were kept in an inescapable condition for 5 minutes, and then removed from the water, dried with towels, and sacrificed immediately via cardiac perfusion following halothane inhalation. Animals were perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH=7.4). After dissection, brains were dehydrated in graded alcohols and embedded in paraffin.

The brain tissue was sectioned at 5 μm thickness and every 30th section, obtained between Bregma 3.00 and 0.60 according to stereotaxic rat atlas by Paxinos and Watson, was mounted on poly-L-lysine coated slides (Figure 1). Deparaffinized and rehydrated sections were treated with 0.3% H2O2 for 30 min, washed in PBS, and then boiled in antigen retrieval solution, containing sodium citrate (pH=6.00) for 5 min in a microwave oven. Unspecific binding was suppressed in a blocking solution for 30 min at room temperature. The rabbit polyclonal antibody to c-Fos (ABE457, Merck Millipore, Darmstadt, Germany) was used at 1:200 dilution. After overnight incubation at 4°C, sections were incubated in biotinylated anti-rabbit secondary antibody (1:100, Novostain Universal Detection Kit, Novocastra Laboratories, Newcastle upon Tyne, UK) for 30 min at room temperature. Bound antibody was detected by streptavidin/ peroxidase complex (PK-6100, ABC Elite Kit, Vector laboratories, Burlingame, CA, USA), and diaminobenzidine (DAB) mixture as chromogen. Finally, sections were dehydrated in alcohol series, cleared in xylene, and coverslipped with permanent mounting medium.
The total number of c-Fos (+) nuclei was quantified using the optical fractionator probe of Stereoinvestigator system (Version 11; Microbrightfield Inc., Williston, VT, USA). Blind-coded slides were investigated by applying systematic random sampling approach in every step. Outlines of the core and shell regions were delineated and all c-Fos (+) profiles within the two-dimensional counting frames (300 μm × 300 μm in size) were quantified under high power magnification. Size of the grid (400 μm × 400 μm × 10 μm) was set to sample 10–15 sites in each section and the optical dissector height was set at 3 μm. The coefficient of error was calculated according to Schmitz and Hof, and values less than 0.05 were considered acceptable.

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess normality distribution of the data. One-way ANOVA followed by Tukey’s multiple comparisons test was used to compare groups. Results were considered significant at a level of p<0.05. IBM SPSS (Statistical Package for the Social Sciences, version 21.0; IBM, Chicago, IL, USA) software was used for all the analyses.

Results
Stress-induced immediate gene expression was evaluated by using c-Fos immunohistochemistry. One-way ANOVA results displayed a significant group difference [F(3,9)=11.04, p=0.0023] in the total number of nucleus accumbens. Total number of c-Fos (+) neurons in PS+SF group (2683×10^6) was significantly (p<0.05) lower than those of CTRL+SF group (5826×10^6), but ketamine injection significantly (p<0.01) enhanced the activation of cells (7.923×10^6) in PS+ketamine group (Figure 2). This activation was also significantly higher than those of CTRL group receiving single dose ketamine injection (p<0.05). The total number of cells in the shell area was also displayed differences among groups [F(3,9)=5.494, p=0.0202]. Total number of c-Fos (+) neurons in PS+SF group (8382×10^5) was significantly (p<0.05) lower than those of CTRL+SF group (3166×10^6), but ketamine injection significantly (p<0.01) enhanced the activation of cells (2937×10^6) only

Figure 1. Representative images displaying core (dark shaded area) and shell (light shaded area) subregions of the nucleus accumbens. Images were taken from “The Rat Brain in Stereotaxic Coordinates” atlas to demonstrate our sampling region between Bregma (a) 3.00 mm, (b) 1.80 mm and (c) 0.60 mm. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]

Figure 2. The total number of c-Fos (+) neurons in the nucleus accumbens. *Indicates significant (p<0.05) difference between saline injected CTRL and PS groups, †indicates significant (p<0.01) difference between PS+SF and PS+ketamine groups. Also, there was a significant difference (p<0.05) between CTRL+ketamine and PS+ketamine groups indicated by + sign. Data shows the mean±SEM of the number of c-Fos expressing neurons per unit area.
in PS+ketamine group. Therefore, the activation of cells was comparable in CTRL groups treated with either ketamine or saline injection (Figure 3).

Likewise the previous groups, activation of cells in the core area displayed a significant group difference [F(3,9)=7.438, p=0.0083]. However, in this subregion of the NAc prenatal stress exposure did not reduce the total number of c-Fos (+) cells. One-way ANOVA results showed that activation of cells in PS group was similar to CTRL groups treated with either SF or ketamine (Figure 4). Thus, group difference was due to enhancement (4986×10^6) in the number of activated cells in ketamine-treated PS group.

Discussion

Stress exposure causes alterations in the morphology and activity of neurons in the mesocorticolimbic structures in a region specific manner. In this study, we showed that PS decreases the number of c-Fos (+) cells in NAc and a single dose ketamine treatment reverses this effect. Interestingly, the impact of prenatal stress exposure was seen only in the shell area, while ketamine treatment affected both core and shell subregions of the NAc.

Previous studies indicate that environmental factors might cause structural, functional, molecular, and epigenetic alterations in the NAc.[28–31] Activation of the ventral hippocampus has an acute stimulatory effect on dopamine release in the shell of the NAC, while it has a suppressive effect on extracellular dopamine levels in the core subregion.[32] Volumetric changes might be seen in the NAc of depressive patients, although inconsistent results were reported in the literature. While most of the studies showed no alterations in the volume of the NAc in patients having major depression,[33,34] some reports displayed reduction in its volume, especially in elderly patients.[35] On the other hand, in glutamate-based depression cases over activation of extrasynaptic NMDA receptors by glutamate causes NAc hypertrophy.[36] In addition, in these patients, an activity reduction could be seen in the whole ventral striatum area.[37,38] Similar to our study’s results, it has been shown that PS modulates the activity of NAc by decreasing the cell proliferation[39] and reducing the volume of NAc.[40] Likewise, stress and depression cause phasic activation of the VTA-NAc pathway and corelease dopamine and BDNF in the NAc region.[41] Ketamine infusion in rodents increases spontaneous activation of dopaminergic neurons in the VTA and extracellular dopamine level in the NAc.[42] These results imply that enhanced neuronal activation seen in the current study following ketamine treatment might be caused by dopaminergic system activation.

It is known that stressful events can induce gene expression by chromatin remodeling and histone modification, which then cause addiction behavior. As Schroeder et al.[43] pointed out, region-specific D1-receptor-regulated histone (phospho) acetylation affects gene expression and differentially regulates reward circuitry. Reus et al.[44] showed that ketamine treatment did not affect histone deacetylase (HDAC) activity in the prefrontal cortex, hippocampus and amygdala of maternally deprived adult rats. In contrast, there is an increased HDAC activity in the nucleus accumbens of deprived rat and it has been shown that ketamine treatment was able to reverse this alteration specifically in this region.
Various NMDA receptor antagonists have been shown to enhance dopamine levels in the NAc in a subregion-specific manner. For example, phencyclidine (PCP) and dizocilpine (MK-801) significantly elevate extracellular dopamine levels in the shell subregion, but not cause alterations in the core subregion. Low doses of another competitive NMDA receptor antagonist CGP 39551 failed to affect dopamine output in either region; while at higher doses it elevates the dopamine output in the shell compared to the core subregion. It seems like, the effects of NMDA receptors and dopamine dysregulation are region specific by affecting predominantly the shell subregion of the NAc. In our study, we observed that ketamine treatment enhanced the activation of cells in both shell and core regions. These differences might be related to the dose and action mechanisms of different NMDA receptor antagonists. As a matter of fact, it has been noted that ketamine acts differentially at higher or lower doses in terms of changing the basal firing rates of ventral tegmental DA neurons.

Conclusion

Current clinical and animal studies report that subanesthetic dose of ketamine produces antidepressant like effect especially in treatment-resistant patients. The effects of ketamine have been mostly investigated following acute or chronic stress exposure, but its effects in prenatal stress paradigm is not well understood. Our study showed that although PS particularly reduces the activation of the cells in the shell subregion, ketamine treatment could act on both core and shell regions by affecting glutamatergic limbic information that flows from shell to core subregion of the NAc.

References


The accessory obturator nerve: an anatomical study with literature analysis

Mehmet Turgut¹, Matthew Protas², Brady Gardner³, Rod J. Oskouian¹, Marios Loukas², R. Shane Tubbs²,³

¹Department of Neurosurgery, Adnan Menderes University School of Medicine, Aydin, Turkey
²Department of Anatomical Sciences, St. George’s University, Grenada, West Indies
³Seattle Science Foundation, Seattle, WA, USA

Abstract

Objectives: The accessory obturator nerve (AON) is often underrepresented in the literature and unknown to many surgeons. As this variant nerve has been mistaken for other regional nerves e.g., obturator nerve, nerve injury has occurred. Therefore, the current study was undertaken to better understand the surgical anatomy of the AON.

Methods: In the supine position, 20 adult fresh frozen cadavers (40 sides) underwent an anterior approach to the retroperitoneal space. When present, the length and diameter of the AON were measured with microcalipers. The position, course and origin of each AON were documented.

Results: The AON was identified on 12 sides (30%). The origin was found to be L2–L3 on four sides; L3 on two sides, L3–L4 from three sides, from the obturator nerve on two sides, and from the femoral nerve on three sides. The average length from the origin to the superior pubic ramus was 14.5 cm. The average diameter was found to be 1.2 mm. All AON were found to lie medial to the psoas major muscle. Additionally, on all sides, the AON was medial to the femoral nerve and lateral to the obturator nerve. Two left sides anastomosed with the anterior division of obturator nerve at its exit from the obturator foramen. Eight sides terminated deep (two) or superficial (six) to the origin of pectineus; two of these had demonstrable branches to the hip joint.

Conclusion: The AON is a normal anatomical variant and there are many variations in its origin and terminal branches can be “strong” or “weak.” Knowing the normal anatomy and variations of the AON is important for surgeons including neurosurgeons, orthopaedic surgeons, and urologists who deal with the pathologies of this area.

Keywords: anatomy; lumbar plexus; posterior abdominal wall; psoas major; variations


Introduction

The accessory obturator nerve (AON) (Figure 1) was first described in 1672 by Isbrand van Diemerbroeck.¹ He reported that it was found in roughly one out of every three persons and originated from the third and fourth lumbar nerves.¹ Not until 1794 was it described in detail by Schmidt (1794). Since its discovery, it has been called the anterior internal crural nerve, accessory nerve of the internal crural nerve, and the nerve of the coxo-femoral articulation.² Some have proposed that it should be named the accessory femoral nerve owing to its typical derivation from the posterior part of the anterior division of L3 and L4, its function, and its anatomical course over the pubic ramus.³

The lumbar plexus is derived from the ventral rami of L1–L4, often with a contribution from T12.⁴ The obturator nerve is most commonly derived from the L2 to L4 ventral rami.⁴ The AON is described in Katritsis et al.⁵ as being derived from the posterior part of the anterior division of L3 and L4. Typically, the AON passes alongside the obturator nerve toward the obturator foramen. Instead of passing through this foramen, it passes over it. There are multiple variations of the terminal branches of the AON after it passes over the superior pubic ramus (Tables 1–3).³⁻⁶

Knowledge of the AON and its variations can be important to surgeons, especially regarding anterior and lateral approaches to the spine.⁶⁻⁹ Misidentification of the nerve can also lead to injury.¹⁰
Materials and Methods

In the supine position, 20 adult fresh frozen cadavers (40 sides) underwent an anterior approach to the retroperitoneal space. Twelve specimens were females and eight were males. Using standard dissection techniques, the abdominal viscera were mobilized and retracted. Once the overlying psoas fascia was identified, it was entered and the psoas major muscle retracted laterally. The obturator nerve was located and the region surrounding it carefully dissected to ascertain the presence of an AON. When present, the length and diameter of the AON were measured with micrcalipers. The position, course and origin of each AON were documented. Statistical analysis between sides and sex were performed with Statistica for Windows (version 10.0; StatSoft Inc., Tulsa, OK, USA) with statistical significance set at p<0.05.

Results

The AON was identified on 12 sides (eight male/ four female) (30%) (seven left/ five right) (two specimens bilateral: both male) (Figure 2). The origin was found to be L2–L3 on four sides; L3 on two sides; L3–L4 from three sides; from the obturator nerve onto two sides; and from the femoral nerve on three sides (Figure 3). The average length from the origin to the superior pubic ramus was 14.5 cm (range: 11–18.5 cm). The average diameter was found to be 1.2 mm (range: 0.8–1.5 mm). All AON were found to lie medial to the psoas major muscle. Additionally, on all sides, the AON was medial to the femoral nerve and lateral to the obturator nerve. Two left sides anastomosed with the anterior division of obturator nerve at its exit from the obturator foramen (Figure 4). This conjoined nerve then traveled as does the normal
obturator nerve course. Eight sides terminated deep (two) or superficial (six) to the origin of pectineus; two of these had demonstrable branches to the hip joint, *i.e.*, piercing the iliofemoral ligament. One side terminated on periosteum of pubic bone (Figure 5). Two AON gave branches to the psoas major within the pelvis (Figure 5). Branches that became very small in caliber and terminated at the superior pubic ramus were termed “weak” branches (Figure 5) and those that were robust and continued beyond the superior pubic ramus as larger branches with multiple branches so of which continued to the obturator nerve where referred to as “strong” branches (Figure 4). No branches to adductor longus were found. No statistical significance was found when analyzing for side or sex.

![Figure 2. Left cadaveric example of an accessory obturator nerve (arrow) arising at the junction of the obturator (ON) and femoral nerves (FN). [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]](image)

---

**Table 1**
Quantitative analysis of each study reporting the prevalence of the AON.

<table>
<thead>
<tr>
<th>Study</th>
<th>Prevalence of AON</th>
<th>Plexus examined</th>
<th>Male prevalence</th>
<th>Female prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eisler (1)</td>
<td>25%</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eisler (1)</td>
<td>29%</td>
<td>120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bardeen (4)</td>
<td>8.4%</td>
<td>250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaiser (3)</td>
<td>8.3%</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Woodburne (5)</td>
<td>8.7%</td>
<td>550</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Webber (6)</td>
<td>8%</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katritsis et al. (5)</td>
<td>13.2%</td>
<td>1000</td>
<td>13.3%</td>
<td>12.9%</td>
</tr>
<tr>
<td>Akkaya (7)</td>
<td>12.5%</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anloague and Hijibregt (8)</td>
<td>8.8%</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>13%</td>
<td>2080</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Table 2**
Sites of origin of the AON.

<table>
<thead>
<tr>
<th>Study</th>
<th>Origin</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quain (14)</td>
<td>Anterior crural nerve</td>
<td>Two cases</td>
</tr>
<tr>
<td>Ellis (15)</td>
<td>Trunk of the obturator nerve</td>
<td>–</td>
</tr>
<tr>
<td>Katritsis et al. (5)</td>
<td>L3-L4</td>
<td>63.60%</td>
</tr>
<tr>
<td></td>
<td>L2-L4</td>
<td>10.60%</td>
</tr>
<tr>
<td></td>
<td>L2-L3</td>
<td>7.60%</td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>6.10%</td>
</tr>
<tr>
<td></td>
<td>Trunk of the obturator nerve</td>
<td>12.10%</td>
</tr>
</tbody>
</table>

---

**Table 3**
Reported terminal branches of the accessory obturator nerve.

<table>
<thead>
<tr>
<th>Study</th>
<th>Terminal branches</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quain (14)</td>
<td>Sensory cutaneous branch of inner thigh</td>
<td></td>
</tr>
<tr>
<td>Allen and Shakespeare (16)</td>
<td>Anastomosing with obturator nerve and supplying skin on inner thigh</td>
<td></td>
</tr>
<tr>
<td>Woodburne (5)</td>
<td>Adductor longus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pectineus muscle sole innervation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pectineus muscle duel innervation (femoral)</td>
<td></td>
</tr>
<tr>
<td>Katritsis et al. (5)</td>
<td>Anterior branch of the obturator nerve</td>
<td>14.30%</td>
</tr>
<tr>
<td></td>
<td>Posterior branch of the obturator nerve</td>
<td>4.65%</td>
</tr>
<tr>
<td></td>
<td>Trunk of the obturator nerve</td>
<td>6.10%</td>
</tr>
<tr>
<td></td>
<td>Femoral nerve</td>
<td>2.30%</td>
</tr>
<tr>
<td></td>
<td>Hip joint</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Prevalence

An AON in humans has a reported incidence of 30%,[21] which is in line with our findings. Other authors have reported an incidence range of 8% to 29%. Bonica[23] reported a prevalence of 8% to 12%. In studies examining the lumbar plexus for the AON, the following was reported: 25% (of Eisler’s 32 plexuses),[6] 29% (of Eisler’s 120 plexuses),[7] 8.4% (21 of Bardeen’s 250 plexuses),[8] 19% (De Sousa et al.),[24] 8.3% (of Kaiser’s 24 plexuses),[9] 8.7% (of Woodburne’s 550 plexuses),[10] 8% (Webber),[11] 11.6% (Sim and Webb),[25] 12.5% (of Akkaya’s 24 plexuses),[12] and 8.8% (of Anloague and Hujibregt’s 30 plexuses).[13] The largest study was conducted by Katritsis et al.[5] in 1980. Among 500 cadavers, the AON was found in 13.2% (13.3% of males and 12.9% of females) with predominance on the left side of the body.[5] Sim and Webb[25] also noted a more frequent occurrence on the left side and in females. Akkaya[12] reported an incidence of three AON in 12 cadavers (four males, eight females). One female patient presented with it bilaterally.

Origin

In our study, the origin of the AON was found to be L2-L3 on four sides; L3 on two sides; L3-L4 from three sides; from the obturator nerve on two sides; and from the femoral nerve on three sides. Katritsis et al.[5] found it to be formed by roots from the anterior primary divisions of L3 and L4 (63.6%) or L2, L3 and L4 (10.6%), or L2 and L3 (7.6%), or L3 (6.1%), or from the trunk of the obturator nerve (12.1%). Ellis[15] found only one case in which the AON arose from the trunk of the obturator nerve. Quain[14] described the origin of the obturator nerve in two cases.

Course

In 100% of the plexuses with an AON examined, Katritsis et al.[5] found that it passed 2–3 cm anterolateral to the obturator nerve and medial to the psoas major towards the obturator foramen, but instead of passing through the canal it passed over the superior pubic ramus, staying medial to the psoas muscle. Woodburne[10] described the AON as passing directly over the pubic ramus under the femoral vein. Various authors have described that once it has passed over the pubic ramus, the nerve descends dorsally to the pectineus muscle, where it typically separates into three branches: one entering the anterior hip joint, one entering the dorsomedial aspect of the pectineus muscle, and one passing medially to anastomose with the anterior branch of the obturator nerve.[10,26] A rare case was reported by Rohini et al.[19] in which the AON divided into
three typical terminal branches, and passed superficially to
the pectineus muscle instead of deep to it.

Multiple variations of the three terminal divisions have
been reported. Katritsis et al.\cite{5} saw that after supplying
the pectineus, the AON branched off behind that muscle, sup-
plying the anterior branch of the obturator nerve (14.3%),
posterior branch of the obturator nerve (4.65%), trunk of
the obturator nerve (6.1%) and femoral nerve (2.3%).
Woodburne\cite{6} also reported that a single branch supply-
ning the adductor longus is not uncommon, along with
other additional branches. A very common variation of the
AON is as the sole innervation of the pectineus muscle,
rather than the typical dual innervation with the femoral
nerve.\cite{14} Quain\cite{14} described a small cutaneous branch that
supplies the inner thigh and upper proximal inner leg.
Allen and Shakespeare\cite{16} reported a similar finding of the
AON anastomosing with the obturator nerve and supply-
ing cutaneous innervation to the skin of the inner thigh. In
one case reported by Tubbs et al.,\cite{27} a pseudoganglion was
found in association with an AON.

Landmarks

Akkaya\cite{12} reported that the mean distance of the AON
from the femoral nerve was 1.6cm, 2.0cm superior and 2.0
cm anterior to the upper wall of the external opening of
the obturator canal, 4.0 cm from the pubic tubercle, and
4.6 cm from the median plane. Although no measure-
ments of the AON have been reported, it is described as
“smaller than the usual obturator nerve”.\cite{10,14,26} Better esti-
mates of the size of the nerve could help in identifying it.

Anatomy

In the past, the prevalence of the AON has consistently
been reported as ranging from 10% to 30%.\cite{10,21,22,5}
Individual studies have involved samples too small for the
prevalence of the AON in the population to be estimated
reliably. When data from previous studies were combined,
the AON was present in 13% (273 of 2,102 plexuses: Table 1).
Most studies failed to record a gender or unilat-
eral bias. However, Sim and Web\cite{25} and Akkaya et al.\cite{12}
reported a greater female and left sided prevalence. These
results could be misleading owing to the paucity of speci-
mens. In the largest study by Katritsis et al.\cite{5} in which
1000 plexuses were examined, there was no apparent dif-
ference between males and females in the prevalence of an
AON in the lumbar plexus. However, there was still a left
sided dominance in unilateral cases. This suggests no asso-
ciation of sided dominance with gender.

Most reports describe the AON as arising from L3 and
L4 (Table 2). Katritsis et al.\cite{4} studied 1000 plexuses (132
plexuses with AON) and found that 36.4% of AON show

Embryology

There are conflicting hypotheses about the embryological
origin of the AON. The original view is that the AON is
derived from a splitting of the obturator nerve caused by
the developing obturator foramen.\cite{10,14,26} This was questioned

Figure 5. Right sided accessory obturator nerve (right arrows) giving rise
to a branch (left arrows) to the psoas major muscle. [Color figure can be
viewed in the online issue, which is available at www.anatomy.org.tr]
by the observation that the pubis develops around the obturator nerve, enclosing it in the obturator foramen. Howell also asserted that when the pubis develops around the obturator nerve, thus separating it from the AON. Yasar et al. reported an AON in four of 20 lumbar plexuses in 10 fetuses between the gestational ages of 24 and 28 weeks. Woodburne described the pectineus as a “border muscle” in embryological development. This is because it is located between the muscles typically innervated by the obturator and femoral nerves. It is located in the anterior thigh but has a function similar to that of an adductor medial thigh muscle. The development of this muscle and its innervation leaves further questions as to whether the AON and its innervation of this border muscle more closely representative of the femoral or obturator nerve. The AON innervates the pectineus on its dorsomedial aspect, while the femoral branch arises distally to the inguinal ligament, turns medially, travels dorsal to the femoral vessels, and finally innervates the muscle on the ventrolateral aspect. With this in mind, the arguments about naming the AON the accessory femoral nerve are questioned by the similar way in which the pectineus in innervated. Bolk described the femoral innervation as frequently being an independent branch and very loosely associated with the femoral nerve. However, Woodburne proposed that the differences in innervation could arise because of phylogenetic separation between the dorsomedial obturator portion of the pectineus muscle and the ventrolateral femoral portion. This raises questions as to whether the phylogenetic separation of the pectineus muscle could account for the embryological development of the AON. In a 14 mm embryo, a distinct branch of the femoral nerve was found to innervate the pectineus. Leche proposed that during development in some mammals, there is an obturator intermedius muscle in addition to the obturator externus. He suggested that this obturator intermedius becomes associated with the pectineus muscle, leading to dual innervation by the femoral nerve and AON. Grafenberg described a six-week-old human embryo in which the muscles of the anterior and medial thigh developed from a single primordial muscle. This primordial muscle was innervated by both the femoral and obturator nerves. Uneven splitting of it could account for the changes in nervous innervation leading to the development of the AON. Visual evidence for this was suggested by Bardeen and Elting, who noticed a mass in association with the embryonic external obturator and pectineus muscles, which he believed to be the area where the obturator nerve would innervate. Evidence supporting this hypothesis is its route and its location on the dorsal aspect of the pectineus muscle after crossing the pelvic brim and before splitting into its terminal branches. Further evidence for the formation of the AON by phylogenetic separation is the atypical path over the pubic ramus (the known path for innervation of the anterior thigh muscles) instead of through the obturator canal.

Clinical Implications
Akkaya et al. reported that the presence of an AON could negatively affect the clinical efficacy of an obturator nerve block. He stated that if the patient has an AON, it could be necessary to block this as well. AON blockage can be recommended for thigh surgeries, treatment of pain, and diagnosis of hip joint pain. Akkaya et al. showed that the location of the AON in 12 cadavers was a mean distance of 4 cm lateral to the pubic tubercle, which should be used as a guide for AON block. Positioning for an AON block should be 2 cm lateral and caudal to the pubic tubercle. The needle should then be rotated 30 degrees lateral and inserted toward the superior edge of the superior pubic ramus. Failure to block the obturator nerve along with the AON completely during a transurethral bladder surgery can lead to life-threatening hemorrhage owing to the proximity of the overextended bladder to an accessory obturator artery. However, Akata et al. were unsure whether the inadequate anesthesia or lack of direct targeting for the AON caused the life-threatening hemorrhage. Akkaya et al. suggested that the best way to prevent injury is to plan for the presence of the AON regardless of the situation to ensure complete obturator nerve blockage.

Conclusion
We studied the AON and documented its origin, course and variants. A better understanding of this nerve’s anatomy can lead to better outcomes following invasive procedures to the area.

Acknowledgements
The authors thank those who donated their bodies for anatomical study. Those were no potential conflicts of interest. Author contributions: Conceived and designed the cadaver study and literature analysis: MT, RJO, RST. Performed the cadaver study: MP, BG. Analysis and interpretation of the data: MT, RJO, ML, RST. Contributed materials/analysis tools: MP, BG, RST. Drafted the article: MT, RST. Approved the final version to be published: MT, MP, BG, RJO, ML, RST. The views expressed herein are those of the authors and not necessarily those of their institutions.
References


15. Ellis H. Demonstrations of anatomy. 11th ed. 1887. a, p. 543; b, p. 631.


This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported (CC BY-NC-ND 3.0) Licence (http://creativecommons.org/licenses/by-nc-nd/3.0/) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Please cite this article as: Turgut M, Protas M, Gardner B, Oskouian RJ, Loukas M, Tubbs RS. The accessory obturator nerve: an anatomical study with literature analysis. Anatomy 2017;11(3):121–127.
Standardization of sternocleidomastoid for botulinum toxin applications*

Bilge İpek Torun¹, Simel Kendir², Aysun Uz²
¹Department of Anatomy, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey
²Department of Anatomy, Faculty of Medicine, Ankara University, Ankara, Turkey

Abstract

Objectives: Botulinum toxin is frequently applied to the sternocleidomastoid muscle (SCM) for torticollis treatment. During this application, bulb of the jugular vein located under SCM makes the interventions unsafe. Also, injecting the botulinum toxin into the infrahyoid muscles which lie under SCM may cause hoarseness and swallowing disorders. The aim of this study was to describe the most reliable and appropriate botulinum toxin injection sites to the SCM to avoid injury to neighboring neurovascular structures and adjacent muscles.

Methods: In ten male cadavers, SCM was evaluated in three equal segments (upper, middle and lower). Muscle width and thickness at the center of each segment were measured. In one male cadaver, colored latex was injected according to the results of the measurements.

Results: The respective mean width of upper, medial and lower segments were 33.15 (23–41) mm, 36.45 (28–45) mm and, 39.35 (15–50) mm, respectively. The mean thickness of upper, medial and lower segments were 5.29 (3.87–7.68) mm, 5.89 (3.56–8.32) mm and 3.60 (0.69–7.75) mm, respectively. There was no significant difference between the right and left sides. The thickest part of the muscle was the middle part, and the lower part was the thinnest. When the colored latex injected cadaver was dissected, the center of the muscle was observed as colored, while the neighboring structures were avoided. The thickest and safest part of SCM for the botulinum toxin injections was the middle part.

Conclusion: Knowing the thickness of SCM will make the botulinum toxin applications to this muscle safer and easier.

Keywords: botulinum toxin; muscle thickness; sternocleidomastoid; swallowing disorder; torticollis

Introduction

Torticollis is the most common form of cervical dystonia presenting short sternocleidomastoid (SCM) with an abnormal head and neck posture.[1] The head tilts towards the short SCM, and the jaw turns to the opposite side. Torticollis is a common disease in all age groups, from newborn to adult.[2]

Successful use of botulinum toxin in the treatment of cervical dystonia has significantly changed the prognosis of patients. In many studies up to now, botulinum toxin has superiority over other pharmacotherapies both in terms of efficacy and less side effects.[1] Botulinum toxin injection into the SCM reduces involuntary head movement and pain causing local muscle weakness.[1]

When botulinum toxin is applied for torticollis, the inferior bulb of the jugular vein just below the SCM makes the interventions dangerous.[1] Furthermore, chemodenervation of the SCM with botulinum toxin may cause dysphagia, effecting the infra- and suprahyoid muscles underlying the SCM. Retrospective studies of the dose and injection site have shown that dysphagia is associated with toxin injection to SCM.[1] According to experts, it is very important for the physician to know the anatomy of the cervical region in order to be able to successfully identify the muscles to be injected.[1]

In this study, we aimed to describe the most reliable and appropriate botulinum toxin injection site of the
Muscle which has no risk of injury to neighboring vascular structures and adjacent muscles.

**Materials and Methods**

This study was performed on formaldehyde fixed 11 adult cadavers from the cadaver collection of Department of Anatomy, Ankara University School of Medicine. The mean age of the cadavers was 55 (range: 24–87). There was no sign of previous surgery or cervical dystonia in the neck region. Ten of these cadavers were bilaterally dissected to expose the SCM. Muscle length, width and thickness of SCM were measured on the dissected cadavers. In one male cadaver, colored latex injection was applied instead of botulinum toxin to muscle segments determined according to the results obtained in this study, bilaterally.

The cadavers were put in supine position, skin and platysma was removed from the underlying facial structures to expose SCM. To measure the length of the SCM, the midpoints of the origin and insertion points were selected. For this, initially the anterior and posterior edges of the SCM were palpated where it inserted on the mastoid process. Midpoint of the insertion side behind the mastoid process (A) was detected. Then a line connecting this point to sternoclavicular joint (B) was drawn, and the distance between A – B points was measured. This distance allowed us to determine the length of the muscle. Then the length of the muscle was divided into three equal parts and the width and the thickness of the center point of each muscle section was measured (Figure 1). We chose the midpoints of each segment to be sure that we measured the width and the thickness of the muscle segments through the same reference point.

The results were statistically evaluated by IBM SPSS (Statistical Package for the Social Sciences, version 20.0; IBM, Chicago, IL, USA) software. According to the results of the study, colored latex was applied on one cadaver, bilaterally. For this procedure, mastoid process (A) the insertion of the SCM and the sternoclavicular joint (B) the origin of the SCM were palpated and marked on the skin. A–B distance was measured. According to the distance, SCM was divided to three equal segments and the mid-points of each segment of the muscle were marked on the skin. Then at the same level, the width of the muscle was palpated and the center of the muscle was marked. Then colored latex was injected into the mid-point of the muscle. Later, the muscles were dissected and then it was observed whether they were colored.

All the measurements were done with a digital caliper sensitive to 0.01 mm and performed by the same observer in the same anatomical position. The results were statistically evaluated by Wilcoxon’s signed rank test.

**Results**

The mean length of the SCM was measured as 188.65 mm (range: 160–220). There was no statistically significant difference between right and left sides. The respective mean widths of upper 1/3, middle 1/3 and lower 1/3 of the muscle were measured as 33.15 (range: 23–41) mm, 36.45 (range: 28–45) mm and 39.35 (range: 15–50) mm, and the thicknesses were 5.29 (range: 3.87–7.68) mm, 5.89 (range: 3.56–8.32) mm and 3.60 (range: 0.69–7.75) mm (Table 1). There was no statistically significant difference between right and left sides.

The results were evaluated statistically and it was found that the ideal site of botulinum toxin injection was...
the middle 1/3 of the muscle, being the thickest part of the muscle and relatively far from the vascular structures. Accordingly, red colored latex instead of botulinum toxin was injected into the center of SCM of one cadaver bilaterally. Than the muscle was dissected and it was observed that the muscle was colored, while the neighboring muscles and veins were not (Figure 2).

Discussion
Currently, botulinum toxin is frequently used for the treatment of some diseases such as strabismus, cervical dystonia, hyperfunctional larynx, pain-headache, temporomandibular diseases, bruxism, and for aesthetic purposes. During botulinum toxin application to SCM for cervical dystonia treatment, complications such as dysphagia, paralysis in neighboring muscles, dry mouth, hoarseness and weakness in neck muscles may be encountered. There are also studies suggesting that spread of toxin to neighboring muscles depends on the amount of toxin given. On the other hand, studies report the spread of toxin to neighboring muscles even in low doses. Thus, applying botulinum toxin to the center of muscle was suggested. Because of the proximity of the SCM with the inferior bulb of jugular vein, there is also risk of injury to this vessel during botulinum toxin application. It is important to know the thickness of SCM so that the procedure can be performed safely.

Studies evaluating the thickness of SCM for botulinum toxin injections are limited in the literature. Hong et al. reported that the thickness of the SCM was less than 1.1 cm from the skin using ultrasound. Although this study does not specify the region where the thickness of the muscle was measured, the results are compatible with our measurements. Also, according to the same study, it was stated that the muscle thicknesses in the patients and the normal group did not differ. Although a group of cervical dystonia patients may develop muscle hypertrophy, it was suggested that muscle atrophy develops due to recurrent botulinum toxin injections.

In an ultrasonographic study performed by Arts et al., the thickness of SCM was reported to differ between males and females, but there was no significant difference between right and left sides. Muscle thickness was measured as 1.01 cm in males and 0.87 cm in females. In this study, muscle thickness was measured from the middle of the muscle and was found thicker than our measurements. The authors noted that the measurements may change in other populations because they selected samples from Western European populations. Likewise, since the samples in our study are from the Turkish population, we are of the opinion that the results may differ according to other populations.

Park et al. found that SCM and internal jugular vein were significantly far from each other in anatomical position when the head is rotated 30° to the opposite side. According to the results of our study, the mid-third of SCM was both a thicker and reliable place for botulinum

<table>
<thead>
<tr>
<th>Mean (mm)</th>
<th>SD</th>
<th>Range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>33.15</td>
<td>23–41</td>
</tr>
<tr>
<td>Middle</td>
<td>36.45</td>
<td>28–45</td>
</tr>
<tr>
<td>Lower</td>
<td>39.35</td>
<td>15–50</td>
</tr>
<tr>
<td>Thickness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>5.29</td>
<td>3.87–7.68</td>
</tr>
<tr>
<td>Middle</td>
<td>5.89</td>
<td>3.56–8.32</td>
</tr>
<tr>
<td>Lower</td>
<td>3.60</td>
<td>0.69–7.75</td>
</tr>
<tr>
<td>Length</td>
<td>188.65</td>
<td>15.52</td>
</tr>
</tbody>
</table>
toxin application, because it is far from the inferior bulb of the jugular vein. We suggest this is a more reliable method when combined with the head rotation described by Park et al.\(^{15}\)

Botulinum toxin blocks acetylcholine release from nerve motor endings and causes the typical flaccid paralysis. Botulinum toxin types are di-chain polypeptides formed by two subunits: heavy chain (H) and light chain (L). The H chain binds to peripheral cholinergic synapses with specific receptors leading to internalization of the L chain. The L chain targets SNARE proteins or synaptobrevin required for the release of acetylcholine into the synaptic cleft irreversibly.\(^{16}\) Animal experiments showed that the closer injection to the motor end point of the muscle’s nerve causes the more effective botulinum toxin application.\(^{19}\) There are studies in the literature about the location of motor end plates on SCM. According to the study of Lee et al.,\(^{20}\) the motor end plates on SCM mostly locate on upper-middle part of the muscle. Combining this knowledge with our measurements, upper and middle segments of SCM could be suggested as the most appropriate parts for botulinum toxin injections. In a study of Delnooz et al.,\(^{21}\) the exact location of the motor end plates of SCM was revealed and they achieved equivalent results in full dose with a half dose of botulinum toxin. When the results obtained in such studies are used together with the results obtained in our study, we think that safer and more effective botulinum toxin applications can be made.

When applying botulinum toxin to SCM, the depth of the muscle also should be known. For this, the thickness of skin on SCM should be measured. Skin thickness varies depending on some factors such as age, gender and body mass index, but can be approximately measured squeezing the skin between two fingers.

The most important limitation of our study was that the measurements were made on formaldehyde-fixed cadavers. It should be kept in mind that there may be 4% loss of tissue when measuring muscle thickness on formaldehyde-fixed cadavers as described by Loukas et al.\(^{22}\) and Apaydin et al.\(^{23}\) Also, the measurements were made on cadavers without cervical dystonia. Although the thickness of the SCM may change in these patients, it is important to keep in mind that the knowledge of normal thickness of the muscle and the planning the interventions accordingly will be helpful in patients with cervical dystonia. One more limitation was that the study was performed on only male cadavers. Studies on also female cadavers will be useful for both increasing the number of measurements and making statistical comparisons.

**Conclusion**

In this study, the length and thickness of SCM was measured in order to perform botulinum toxin applications safely. The mean length of SCM was found as 188.65 mm, the thickest part of the muscle was shown as the middle third as 5.89 mm, and the thinnest part was the lower third as 3.6 mm. We suggest that the middle part of the muscle is the safest part for botulinum toxin applications, because it is the thickest part and has relatively far distance from the inferior bulb of the jugular vein. Since the lower third of the muscle is the closest part to the inferior bulb of jugular vein and since it is the thinnest part of the muscle, we recommend avoiding botulinum toxin applications to this area.

As conclusion, knowing the thickness of SCM throughout its course according to the measurements obtained in our study will make the botulinum toxin applications to this muscle safer and easier.

**References**


Online available at: www.anatomy.org.tr
doi:10.2399/ana.17.039
QR code:

Correspondence to: Bilge Ipek Torun, MD
Department of Anatomy, Faculty of Medicine,
Yıldırım Beyazıt University, Ankara, Turkey
Phone: +90 532 246 87 78
e-mail: bilgeipek@yahoo.com

Conflict of interest statement: No conflicts declared.
The zona orbicularis of the hip joint: anatomical study and review of the literature

Alexandra Fayne\textsuperscript{1}, Peter Collin\textsuperscript{2}, Melissa Duran\textsuperscript{1}, Helena Kennedy\textsuperscript{2}, Kiran Matthews\textsuperscript{3}, R. Shane Tubbs\textsuperscript{4,5}, Anthony V. D’Antoni\textsuperscript{6}

\textsuperscript{1}SUNY Downstate College of Medicine, New York, USA
\textsuperscript{2}New York University School of Medicine, New York, USA
\textsuperscript{3}City University New York, New York, USA
\textsuperscript{4}Seattle Science Foundation, Seattle, WA, USA
\textsuperscript{5}Department of Anatomical Sciences, St. George’s University, Grenada, West Indies
\textsuperscript{6}Division of Anatomy, Department of Radiology, Weill Cornell Medical College, New York, USA

Abstract

Objectives: Although it is used as a landmark during various orthopedic procedures of the hip, few studies have focused on the anatomy of the zona orbicularis. Therefore, the purpose of the present research was to study its morphology to improve our understanding of its structure and potential variation.

Methods: Ten adult cadavers (four males and six females) underwent dissection of the left and right hip joints to observe the morphology and location of the zona orbicularis. A digital caliper was used to measure the length and width of the zona orbicularis.

Results: We found a zona orbicularis on all sides and that when present anteriorly, many of the inferior fibers of the zona orbicularis were confluent with the fibers of the iliofemoral ligament. The mean length for right sides was 35.95 mm and the mean length for left sides was 43.93 mm. The mean width for right sides was 3.74 mm and the mean width for left sides was 4.4 mm. There were no significant differences between the right and left sides for zona orbicularis length or width. There was no significant association between age and sex but a statistically significant correlation ($r=0.959$) between right and left zona orbicularis lengths ($p=0.041$). However, there was no significant correlation between the right and left zona orbicularis widths ($p>0.05$). The fibers of the zona orbicularis were found to be thicker and more visible along the posterior aspect of the femoral neck.

Conclusion: Anatomically, the zona orbicularis is a consistent structure of the hip joint capsule. This structure can be seen on high resolution imaging such as MRI and magnetic resonance arthrography. The lengths of this structure may differ between sides, although based on our study, there were no differences between left and right side widths or between sexes or among ages.

Keywords: anatomy; capsule; hip joint; ligaments, surgery

Anatomy 2017;11(3):133–136 ©2017 Turkish Society of Anatomy and Clinical Anatomy (TSACA)

Introduction

The zona orbicularis is one of the ligaments that constitute the capsular hip joint.\textsuperscript{[1]} It lies underneath the synovial capsule with the fibers located in a horseshoe or circumferential pattern around the femoral neck.\textsuperscript{[2,3]} The fibers are most abundant posteriorly and inferiorly and move in a posterolateral to anteromedial fashion, placing them exactly perpendicular to the femoral neck (Figure 1). Anteriorly, they blend with the iliofemoral ligament, while superiorly the zona orbicularis blends with the ischiofemoral ligament and connects to the base of the greater trochanter.\textsuperscript{[4–6]} As it moves inferiorly, the zona orbicularis is twisted from posterolateral to anteromedial. The zona orbicularis, once called the anular ligament of the hip, orbicular zone, ring ligament and zonular band, has been hypothesized to contribute to hip joint stability and resistance to distraction of the hip.\textsuperscript{[5–7]}

Surgically, the zona orbicularis is used as a landmark. In arthroscopic procedures, it is used as a landmark in lateral approaches to the hip for repair of peripheral compartment
pathologies.\textsuperscript{[3,8–10]} In anterolateral approaches, the zona orbicularis is used as a landmark to avoid injury to the branches of the lateral circumflex femoral arteries. Such injuries can disrupt blood flow to the neck and head of the femur.\textsuperscript{[9]} In order to relieve the pain from a symptomatic internal snapping hip, an arthroscopic approach can be pursued after all medical and conservative measures have failed. This procedure uses an anterior portal approach to release the iliopsoas tendon. In order to visualize the tendon, the fibers of the zona orbicularis are used as a landmark.\textsuperscript{[10]}

Few studies have focused on the anatomy of the zona orbicularis.\textsuperscript{[4,13]} Therefore, the purpose of the present research was to study the morphology of the zona orbicularis and its anatomical variation to improve our understanding of its structure.

**Materials and Methods**

Ten embalmed adult cadavers (four males and six females) underwent dissection of the left and right hip joints using blunt and sharp dissection. Specifically, the dissections focused on observing the morphology and location of the zona orbicularis. The mean (SD) age at death of the specimens was 82.1 (14.5) years with a range from 54 to 98 years.

For anterior dissections, the cadavers were placed supine. The origins of the sartorius, rectus femoris, and pectineus muscles were reflected inferiorly to expose the hip joint. The femoral vessels and nerves were then cut to reach the iliopsoas muscle and tendon, which was removed from the lesser trochanter of the femur to expose the joint capsule. At this point, the iliofemoral ligament and pubofemoral ligament were visible anteriorly.

In the prone position, the gluteus maximus was dissected; the medius and minimus were reflected inferiorly from their insertions on the greater trochanter of the femur. The piriformis, obturator internus, gemelli, quadratus femoris, and obturator externus muscles were cut from their superior attachments to reveal the posterior aspect of the fibrous capsule of the hip joint.

Next, an incision was made spanning medial to lateral along the more proximal and superior aspect of the anterior head of the femur. Care was taken to avoid the neck of the femur. The cut was made deeply in order to separate the femoral head completely from the capsule. Blunt dissection was then used to remove the rest of the capsule inferiorly to expose the zona orbicularis completely. Photographs were taken of the anatomical dissections. A digital caliper was used to measure the length and width of the zona orbicularis. Data were entered into SPSS version 22 for statistical calculations with statistical significance set at \( p<0.05 \).

**Results**

When present anteriorly, many of the inferior fibers of the zona orbicularis were confluent with the fibers of the zona orbicularis. Table 1 shows the measurements of the mean lengths and widths of the zona orbicularis. There were no significant differences between the means of the right and left sides for zona orbicularis length or width. The ranges for width for left and right sides was 3.17–6.32 mm and 2.03–4.61 mm, respectively. The ranges for length for left and right sides was 31.43–70.54 mm and 32.64–44.61 mm, respectively. There was no significant association between age and sex using a Mann-Whitney U test (\( p>0.05 \)). A paired sample corre-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Measurements of the zona orbicularis.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
</tr>
<tr>
<td></td>
<td>Right sides (mm)</td>
</tr>
<tr>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>Length</td>
<td>35.95 (2.9)</td>
</tr>
<tr>
<td>Width</td>
<td>3.74 (0.4)</td>
</tr>
</tbody>
</table>

SE: standard error
lation demonstrated a statistically significant correlation ($r=0.959$) between right and left zona orbicularis lengths ($p=0.041$) ([Figures 2 and 3]) but no significant correlation between right and left zona orbicularis widths ($p>0.05$). The zona orbicularis fibers were found to be thicker and more visible along the posterior aspect of the femoral neck.

**Discussion**

We found a zona orbicularis on all sides and when present anteriorly, many of the inferior fibers of the zona orbicularis were confluent with the fibers of the iliofemoral ligament. The mean length for right sides was 35.95 mm and the mean length for left sides was 43.93 mm. The mean width for right sides was 3.74 mm and the mean width for left sides was 4.4 mm. There were no significant differences between right and left sides for zona orbicularis length or width. There was no significant association between age and sex but a statistically significant correlation ($r=0.959$) between right and left zona orbicularis lengths ($p=0.041$). However, there was no significant correlation between right and left zona orbicularis widths ($p>0.05$). The fibers of the zona orbicularis were confluent with the fibers of the iliofemoral ligament. The mean length for right sides was 35.95 mm and the mean length for left sides was 43.93 mm. The mean width for right sides was 3.74 mm and the mean width for left sides was 4.4 mm. There were no significant differences between right and left sides for zona orbicularis length or width. There was no significant association between age and sex but a statistically significant correlation ($r=0.959$) between right and left zona orbicularis lengths ($p=0.041$). However, there was no significant correlation between right and left zona orbicularis widths ($p>0.05$). The fibers of the zona orbicularis were confluent with the fibers of the iliofemoral ligament.
orbicularis were found to be thicker and more visible along the posterior aspect of the femoral neck.

Using magnetic resonance arthrography, Malagelada et al. found that the zona orbicularis was absent anteriorly on the majority of sides. Using MRI, it was absent posteriorly on roughly half of sides, absent inferiorly on roughly one third of sides and anteriorly on 8% of sides. Their study also pointed out that the zona orbicularis was often congruent with the periphery of the head of the femur and its narrowest point was at the isthmus of the neck of the femur. Malagelada et al. concluded that the zona orbicularis is most consistently identified when the joint is distended and that it is aligned perpendicular to the long axis of the neck of the femur. These authors also supported the hypothesis that the zona orbicularis functions as a gasket that resists the distraction of the head of the femur and thus contributes to synovial fluid circulation.

Ito et al. hypothesized and then concluded that the zona orbicularis functions in resisting hip distraction. In seven cadaveric hips, in a direction parallel to the body of the femur, the hip joint was distracted. Next, the overlying soft tissues were sequentially dissected: (1) intact specimen (muscle and skin removed), (2) capsule opened, (3) iliofemoral ligament cut, (4) circumferentially capsule incised, (5) partially resected capsule, (6) completely resected capsule, (7) radially incised acetabular labrum, and (8) completely resected acetabular labrum. The reduction of the distraction load was greatest between the partially resected capsule phase and completely resected capsule phase at 1, 3, and 5 mm joint distraction.

**Conclusion**

Anatomically, the zona orbicularis is a consistent structure of the hip joint capsule. This structure can be seen on high resolution imaging such as MRI and magnetic resonance arthrography. The lengths of this structure may differ between sides although based on our study, there were no differences between left and right sided widths or between sexes or among ages.

**Acknowledgements**

The authors thank those who donated their bodies for medical study. Also, the authors thank the following individuals for their assistance in the dissections: Orin Imani Donaldson, Samantha Syldort, and Melissa Evelyn.

**References**

Bologna Process: elective lectures and internships in medical curriculum and the role of anatomy electives

Mustafa F. Sargon

Department of Anatomy, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Abstract

Objectives: Due to objectives of Bologna Process, updated programs of School of Medicine, Hacettepe University were put into practice in 2013–2014 academic years. Progress of elective lectures and internships in undergraduate medical education and the role of anatomy were discussed. The outcomes of all data were obtained from the experiences of three academic years.

Methods: Before Bologna Process, electives were present in the curriculums of Phase I and VI. Beginning with this process; two elective committees were added to curriculums of Phase I–III. In Phase IV and V, electives were organized as being part of internship. The new approach to elective lectures and internships were discussed both from point of medical students and lecturers.

Results: Phase I-III students showed two major tendencies: choosing elective lectures related to medicine and choosing the ones which were in same campus. They also had two major complaints: difficulty of some electives and some undesired electives due to packaging system or related to extreme number of students. From the point of anatomy, students preferred to choose anatomy electives which were useful for anatomy curriculum. Lecturers complained from the extreme number of students, low number of electives and decreased number of theoretical hours. The extreme number of students and low number of electives seemed the major problems. Therefore, decreasing the number of elective lectures can be a solution. Additionally, the remaining time from these electives can be added to basic medical lectures.

Conclusion: Excited lecturers with ideal number of students can perform the best education. From the point of anatomy, many elective clinical anatomy lectures are essential for drawing the attention.

Keywords: anatomy electives; Bologna Process; elective lectures; medical curriculum, undergraduate medical education

Introduction

The European Ministers responsible for higher education in 29 countries were gathered in Bologna in June 19, 1999 and signed the Bologna declaration. They agreed on the development of a consistent and harmonious European Higher Education Area by 2010. One of the basic purposes for the European Higher Education Area was to strengthen the international competitiveness of European higher education for the undergraduate, postgraduate and doctoral graduate studies which have precisely defined duration.

Due to the objectives of Bologna Process, the updated programs of School of Medicine, Hacettepe University were put into practice in 2013–2014 academic years. One of the major changes in these programs was the addition of elective lectures and elective internships to the medical curriculum of undergraduate students of all six years (twelve semesters). In order to add these electives to the medical curriculum of undergraduate students, the lecture and practical hours of basic lectures were decreased 20% in all of the semesters.

The present study aimed to compare the updated programs of Bologna Process and the previous medical curriculum from the point of elective lectures in School of Medicine, Hacettepe University. The comparison was done both from the perspectives of medical students and
lecturers in an experience of 2013–2014, 2014–2015 and 2015–2016 academic years. Related to these programs, progress of elective lectures and internships in undergraduate medical education were examined in the study. Additionally, the demands of undergraduate students to anatomy elective lectures were discussed.

Materials and Methods
In School of Medicine, Hacettepe University medical curriculum, elective lectures were present in the curriculum of Phase I throughout the entire academic year and in Phase VI till the end of the academic year 2012–2013. Before the beginning of 2013–2014 academic years, the medical curriculum was fully updated in accordance with the updated programs of Bologna Process. Two elective committees were added to the curriculums of Phase I, Phase II and Phase III. Each of these committees consisted of four elective lectures which were brought together to form a package. While making a package by these four elective lectures, a great care was shown for making the packages from the elective lectures of different departments. In every committee, 75% of these lectures (totally three lectures in each committee) were given by the lecturers of the Medical Faculty of Hacettepe University. The remaining 25% of elective lectures in each committee (one lecture) was given by the Faculty of Education of Hacettepe University. The medical students' major complaint about the lectures of Faculty of Education was the lecture halls which were situated in the other campus of the university. Therefore, the complaints of the students were taken into consideration and the lecturers were invited to the campus of Medical School lecture halls.

In the medical curricula of Phase IV and Phase V, there were no elective lectures before the updated programs of Bologna Process. Beginning from the academic year of 2013–2014, the electives were organized as being a part of internship and added to the final two weeks of the training program in Phase IV. These elective lectures were directly related with the present internship program and they aimed to give some detailed knowledge and practical applications related to the present internship program. In Phase V, three elective lectures were added to the medical curriculum and each of them was organized to be three weeks. The Bologna Process did not bring any change to the medical curriculum of Phase VI in School of Medicine, Hacettepe University. They still had two electives and medical students prefer one elective from medical sciences and the other one from surgical sciences divisions.

Results
In School of Medicine, Hacettepe University, in Phase I, Phase II and Phase III, the period of elective lecture packages were totally four weeks. Every week, each elective lecture had a lecture hour of four hours, and therefore, totally, each elective lecture had a total of 16 lecture hours in the committee. Additionally, due to the four elective lectures in a package; in every semester; the total lecture hours of electives were 64 hours in each elective committee.

The Phase I, Phase II and Phase III students showed two major tendencies: choosing the elective lectures related to medicine and choosing the ones which were given in the same campus of the university. Therefore, beginning from the first day of 2013–2014 academic year, all of the elective lectures were given in the lecture halls of the Medical School. They also had two major complaints: the difficulty of some elective lectures and some undesired elective lectures due to the packaging system of four elective lectures together. The extreme number of students (around 60 students in each package) was another undesired condition for the electives both from the point of undergraduate students and lecturers.

In Phase IV and V, the students did not have any complaints about the ongoing of the elective internships. In Phase IV, their only complaint was about the exam of the internship program. The medical students attended to two different oral examinations during their internship program. The first oral exam was done at the end of the internship program and the second was done at the end of the elective part of the internship program. Both of these two oral exams were independent from each other. In Phase V, the students attended to the exam of each elective lecture at the end of each internship. The Phase VI students did not have any complaints about the elective internship programs in their medical curriculum.

From the point of lecturers of Phase I, Phase II and Phase III, the major complaint was the extreme number of students and low number of elective lectures. These lecturers had many choices for grading at the end of the electives. Preparing test questions, giving home works and attendance can all or only one be used for grading in these three years. In Phase IV and Phase V, the only method of grading was done by the exams. In Phase VI, the students were graded according to their hard working at the end of the internship program. Additionally, the common complaint of most lecturers of Phase I-V was the decreased number of theoretical hours.

Anatomy elective lectures took part in the elective committees of Phase I and Phase II medical curricula.
Phase II undergraduate medical students prefer clinical anatomy elective lectures and they tried to make contribution to these lectures. Problem solving about the clinical condition is the part of these lectures which they really were interested. Interestingly, many undergraduate medical students also requested some more problem solving lecture hours related with the other subjects’ clinical anatomical conditions.

Discussion
In order to become a good doctor, finishing a medical school is not enough. A picture of a doctor involves highly educated person who invested a lot in himself and his education. Therefore, there is a great effort for revealing the best medical education methods in all over the world. The university staffs who devote their lives to better medical education discuss new methodologies and try to find the best one for their precious students.

Elective lectures and elective courses are an excellent way to increase students’ exposure to special areas which they really are interested. Zapantis et al.[9] designed an elective course in adult acute care medicine using a hybrid delivery system. They concluded that the course increased student exposure to inpatient settings and provided students additional opportunities to communicate effectively, evaluate medical literature and think critically. Marshall and Ashworth[10] developed a women’s health course focusing on health promotion, disease prevention and treatment throughout a woman’s life span. At the end of the course; excellent student performance in weekly active-learning activities and class participation was observed. However, this did not translate into excellent performance on subsequent formal assessments. Zalihic and Obredaj[11] organized a minor elective course entitled “communication skills” to medical students and discussed the importance of the subject in doctor-patient therapeutic relationship. Mouradian et al.[12] developed an oral health elective that targeted first and second year medical students as part of a previously described oral health initiative and oral health curriculum. Evaluations revealed positive shifts in attitudes toward oral health and significant gains in oral health knowledge and self-confidence. At the end of the elective; the medical students rated the course highly and advocated for further integration of oral health into required medical curricula. Eighteen second year medical students participated in the preclerkship HIV elective consisting of lectures, small group sessions, clinical observership, community placements, reading assignments and an HIV counseling and testing workshop. Self-assessment scores of HIV knowledge among the students significantly increased from 78.1% (prior to elective) to 90.2% (at the end of the elective).[13] Caro-Bruce et al.[14] organized a collaborative and multidisciplinary elective course related with the gaps in the areas of abortion and sexual health to a group of first and second year medical students. The elective proved to be a successful collaboration among students, faculty, healthcare providers and resulted in permanent changes in the standard medical school curriculum.

The Bologna Process brought a new approach to medical education. In the study, the effects of Bologna Process to the medical education system of School of Medicine, Hacettepe University was discussed from the point of elective lectures. The outcomes of these data were obtained from the experiences of three academic years. During these three academic years, more than 2500 Phase I – Phase VI medical students’ and more than 100 lecturers’ opinions and contributions were also discussed. The elective courses must have diversity. Not only the medical elective courses, but also the elective courses of different scientific areas must also be added to medical curriculum. Medical doctors who are highly educated persons must also have a good knowledge about the other scientific areas which they are really interested in. The diversity of the medical and non-medical elective lectures is very important from that point of view.

The number of students who are attending to an elective course is very important. The aim of the elective must be well known and the organization must be perfect prior to lecture or course. In the cases of extreme number of students and low number of electives, it is better to diminish the number of electives which will be taken by the students. This will decrease the number of students per elective lecture and will help to the goals of the course. Additionally, the remaining lecture hours from these electives can be added to the basic medical curriculum. This solution will make the lecturers who are against Bologna Process to feel better. In medical schools with extreme number of students; giving a support to basic medical curriculum will develop the quality of medical education.

Another important point about the electives is their contribution to the grading system. It can be organized freely by each medical school and must always include a less contribution than the basic medical lectures.

The anatomy elective courses seemed very popular among undergraduate medical students. The students require more and more clinical anatomy from the lecturers. However, the adequate number of students for each clinical anatomy elective course must be considered by the lecturers. Additionally, it is ideal to add role modelling to the contents of the courses. Bologna Process and new electives bring a new approach to anatomy and make it very popular among the undergraduate medical students.
Conclusion
Medical curriculum investigates new methodologies and new approaches in order to graduate perfect medical doctors. One can never describe the best method in medical education. The number of undergraduate medical students is also a very important factor in medical education and depending upon to it; the medical faculties can choose the best method for their education system. In conclusion, hardworking and excited lecturers combined with ideal number of students can perform the best medical education with any of the methodologies.

Evaluating the Bologna Process and elective courses from the point of anatomy; various elective clinical anatomy courses are essential for drawing the attention. However, the adequate number of students for each clinical anatomy elective course must be considered by the lecturers. Bologna Process and new electives bring a new approach to anatomy and make it very popular among the undergraduate medical students.

References

Correspondence to: Mustafa F. Sargon, MD
Department of Anatomy, Faculty of Medicine, Hacettepe University, Ankara, Turkey
Phone: +90 532 246 87 78
e-mail: mfsargon@hacettepe.edu.tr
Conflict of interest statement: No conflicts declared.
Introduction

Injuries to the atlanto-occipital joint (AOJ) (Figure 1) make up 15–30% of all cervical spine injuries and 19% of such injuries are fatal. These injuries are more common in children due to the laxity of surrounding ligaments around the AOJ. The AOJ connects the occipital condyles (OC) (Figure 2) and the superior articular surfaces (SAS) (Figure 3) of the lateral mass of the atlas. The mean length, width, and height of the OC are 23.6 mm (a range of 16.7–30.6 mm), 10.5 mm (a range of 6.5–15.8 mm), and 9.2 mm (a range of 5.8–18.2 mm), respectively. The long condylar axes converge medially, forming the sagittal intercondylar angle, which ranges from 22 to 103° (mean of 59.3°). The mean distance between the resultant anterior ends of the OCs, which is closer to the midline, is 23.6 mm (a range of 16–30 mm) and that of the posterior ends (Figure 4), which are located just anterior to the equator of the foramen magnum, is 42.1 mm (a range of 37–50 mm). The OCs can be categorized according to their various shapes such as oval in 50% of all cases, S-shaped in 23.2%, and triangular in 9%. Another classification is possible with the lengths of the OC, which is 26 mm in 77.2% of the cases: short OCs (less than 20 mm) in 8.6% and long OCs (greater than 26 mm) in 14.1%. The alar ligament attaches to the medial surface of the OC.

The atlas is made up of two lateral masses, which are connected together by an anterior arch and a posterior arch. The lateral masses bear the weight of the cranium. The SAS of the atlas is concave (Figure 5), forming a framework for the convex OCs, and is on the superior aspect of the lateral mass, which is trapezoid in shape and higher laterally (22 mm; a range of 17.5–28.5 mm) than it is medially (11 mm; a range of 8.2–14.6 mm). The SAS slopes medially with a mean angle of 22.4° (a range of 16.5–29.2°) and converges medially with a mean horizontal angle of 18.6 mm (a range of 15.5–21.8 mm). The surface of the AOJ has a C-shape and is concave medially. The AOJ is reinforced by a joint capsule.

Atlanto-Occipital Ligaments

The ligamentum nuchae (LN) is a continuation of the supraspinous ligament, which spans from the occipital protuberance to the vertebra prominens (C7). The LN...
limits excessive neck flexion.\textsuperscript{[5]} The alar ligament (AL) (Figure 6) limits axial rotation and lateral flexion, stabilizing the AOJ. The AL is the primary ligament for the stabilization of the joint when the transverse ligament is damaged.\textsuperscript{[5]} The AL also anchors the dens to the OCs and is very tough.\textsuperscript{[6]} The anterior and posterior atlanto-occipital membranes attach to their respective surfaces on the atlas and the foramen magnum.\textsuperscript{[5]} The apical ligament (Figure 6) joins the tip of the odontoid process to the basion (the most anterior point of the foramen magnum in the median sagittal plane) and has no mechanical role and is absent in 20% of the bodies studied by Tubbs et al.\textsuperscript{[5]} The AOJ allows 11–13° of freedom, allowing for 25° of flexion and extension, 5° of axial rotation, and combined motions.\textsuperscript{[7]} Flexion is limited by the contact between the foramen magnum and the dens.\textsuperscript{[1]}

According to Tubbs et al., the tectorial membrane (TM) (Figure 6), a mean thickness of 1 mm, firmly attaches cranial base and body of the axis.\textsuperscript{[8]} The TM stabilizes the head in flexion and extension of the neck.\textsuperscript{[8]} It also carries a function of limiting the posterior movement of the odontoid process and prevents the process from intruding into the cervical canal and compress the spinal cord.\textsuperscript{[8]}

The lateral atlanto-occipital ligament (LAO) is situated right against the rectus capitis lateralis bilaterally and is
in a close contact with the jugular foramen anteriorly and the vertebral artery posteriorly.\textsuperscript{[9]} The ligament originates from the anterolateral aspect of the transverse process of the atlas and inserts onto the jugular process of the occiput.\textsuperscript{[9]} LAO has a mean angle of $26^\circ$ from the midline and a mean length and width of 2.2 and 0.5 cm, respectively.\textsuperscript{[9]} This ligament limits lateral flexion of the neck and partially limits neck rotations bilaterally at the AOJ.\textsuperscript{[9]}

The alar ligaments (AL) (\textbf{Figure 6}) are dense structures that connect the odontoid process to the medial aspect of the OCs.\textsuperscript{[10]} The ALs join the superior half of the lateral aspect of the dens and the medial surface of the OC, at which the distal insertions of the ligaments are limited.\textsuperscript{[10]} They limit the axial rotation, lateral flexion, and sagittal flexion at the AOJ. The alar ligaments are some of the most important structures around the AOJ, which not only protects but also allows mobility of the neurovascular bundles which run within the cervico-cranial junction.\textsuperscript{[10]}

\section*{Injuries to the AOJ}

In a traumatic injury involving the AOJ, its supporting ligaments may be torn and dislocate the atlas from the occiput.\textsuperscript{[10]} Such an injury is classically associated with a high-energy impact trauma in a road traffic accident or a fall from a height.\textsuperscript{[11]} According to Horn et al., the presence of an atlanto-occipital dislocation must be assessed in all traumatic brain injuries.\textsuperscript{[12]} Most cases of dislocation require immediate stabilization.\textsuperscript{[12]}

There are two types of atlanto-occipital injuries: atlanto-occipital dislocation (\textbf{Figure 1}) and OC fractures. A damage to the ligaments in this region may cause vary-
ing degrees of atlanto-occipital instability and subluxation or luxation of the joint between the atlas and the axis.\textsuperscript{[13]}

Traynelis et al. classified atlanto-occipital injuries according to the direction of the dislocation: type 1 – anterior dislocation, type 2 – vertical displacement, and type 3 – posterior dislocation.\textsuperscript{[13]}

The radiologic diagnosis of atlanto-occipital injuries is challenging. The basion to dens interval (BDI), the distance between the basion and the tip of the dens, and the basion to axial interval (BAI), the distance between the basion and a line drawn at the posterior border of the body of the C2 and dens extended cranially, should be less than 12 mm in a lateral cervical radiograph to rule out an atlanto-occipital dissociation.\textsuperscript{[14]}

The intraarticular fluid sign is shown by the magnetic resonance imaging (MRI) and indicates a disrupted AOJ capsule and an injury to the surrounding ligaments.\textsuperscript{[15]} The revised OC to C1 interval (CCI) shown by the computed tomography (CT) is highly sensitive and specific for an atlanto-occipital injury and is measured from the bottom of the OC to the deepest point in the valley of the SAS of C1 in the parasagittal plane.\textsuperscript{[15]} A CCI of greater than 2.5 mm is considered diagnostic of unilateral or bilateral AOJ dislocation.\textsuperscript{[15]}

A fracture of the OC is relatively rare and two classification systems are utilized. Anderson and Montesano developed three categories of OC fractures: type I – impaction fracture of the OC, which is considered stable, type II – fracture of the skull base in which the fracture line crosses the OC, type III – avulsion fracture of the OC resulting in ligamentous instability.\textsuperscript{[16]} Types I and II fractures are stable while type III fracture is unstable.\textsuperscript{[16]} Tuli et al.\textsuperscript{[17]} reported a classification system in regards to the presence of dislocation, which is defined by more than 2 mm of separation, or occipito-atlantoaxial instability: type 1 – OC fracture without dislocation and type 2 – dislocated OC fracture. Type 2 is further subdivided into 2a, which is a case of a stable occipito-atlantoaxial complex, and 2b, which is a case with any signs of occipito-atlantoaxial instability. A surgery is required only in cases of presence of an instability or a compression of the neural structures, both of which are rare.\textsuperscript{[17]}

**Conclusion**

The AOJ (Figures 7 and 8) is the articulation point of the OC and the SAS of the atlas. The structure of this joint is maintained and reinforced by multiple surrounding ligaments, which allow the spinal cord to pass through the foramen magnum without any bony interference. Contact sports and high-impact collisions are the common mechanisms of injury to the AOJ and may be fatal. A timely diagnosis of AOJ injuries increases the likelihood of survival.\textsuperscript{[15]} In addition, subluxations can also be caused by ligament laxity and muscular atrophy in osteoarthritis.\textsuperscript{[15]}

![Figure 7. Posterior view of the craniocervical junction noting the articulation between the occipital condyles and superior articular facets of C1. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]](image)

![Figure 8. Lateral view of the craniocervical junction noting the articulation between the occipital condyles and superior articular facets of C1. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]](image)
References

Correspondence to: Paul J. Choi, MD
Seattle Science Foundation, 550 17th Ave, James Tower, Suite 600, Seattle, WA 98122, USA
Phone: +1 206 732 65 00
e-mail: paulchoi92@gmail.com
Conflict of interest statement: No conflicts declared.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported (CC BY-NC-ND3.0) Licence (http://creativecommons.org/licenses/by-nc-nd/3.0/) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Please cite this article as: Bodon G, Choi PJ, Iwanaga J, Tubbs RS. The atlanto-occipital joint: a concise review of its anatomy and injury. Anatomy 2017;11(3):141–145.
The iliocapsularis muscle (Figure 1), also referred to as the iliacus minor or iliotrochantericus, is a muscle over the human hip just deep to the rectus femoris. It originates from the anterior inferior iliac spine and the anteromedial hip joint capsule and inserts on to the lesser trochanter. \[1–4\]

Although the iliocapsularis is apparently constant in humans, the literature fails to describe it in detail. Interestingly and comparatively, it has been found in primates, rats, reptiles, and birds.\[1\]

Although the anatomy of the iliocapsularis has been described, its true function is disputed. Multiple functions have been proposed and supported in the literature. Additionally, the iliocapsularis has been used as an important surgical landmark in procedures involving the hip joint capsule. Moreover, studies have shown that understanding its radiological appearance is important in categorizing pathologies of the hip (e.g., hip dysplasia).

Functional Relevance

Although hypotheses have been proposed, the true function of the iliocapsularis has not been elucidated. It was first mentioned in 1843 by the French anatomist Jean Cruveilhier, who suspected the muscle acted as a stabilizer to the femoral head; however, he offered no explanation as to how this might be achieved.\[5,6\] Some propose that the iliocapsularis helps to prevent synovial impingement, a possibility supported by electromyography studies. Moreover, hypertrophy of the iliocapsularis caused by hip dysplasia supports the proposal that it stabilizes the anterior aspect of the joint capsule. This review discusses the clinical relevance of the iliocapsularis.

Abstract

The iliocapsularis muscle covers the anterior aspect of the hip joint capsule. Although its anatomy has been described through cadaveric and radiologic studies, its true function is unknown. The iliocapsularis could be involved in preventing anterior synovial impingement, a possibility supported by electromyography studies. Moreover, hypertrophy of the iliocapsularis caused by hip dysplasia supports the proposal that it stabilizes the anterior aspect of the joint capsule. This review discusses the clinical relevance of the iliocapsularis.

Keywords: hip dysplasia; iliocapsularis; iliacus minor; iliotrochantericus; rectus femoris; stability
Because of the deep location of the iliocapsularis, electromyography (EMG) has only recently been successfully recorded for it. EMG provides insight into muscle activation, which can potentially elucidate muscle function. Lawrenson and colleagues placed EMG electrodes in the iliocapsularis under ultrasound guidance. The electrode was passed through the sartorius muscle, lateral to the femoral artery and medial to tendon of the rectus femoris. Coursing in a slightly medial direction, the electrode then penetrated the iliacus and finally the iliocapsularis. The maximum iliocapsularis activity was documented during resistance to hip flexion >90°, and the minimum during hip extension from the neutral position (0°).

As previously mentioned, one proposed function of the iliocapsularis is to prevent synovial impingement during flexion. The maximal EMG activity during hip flexion at >90° supports this proposal. The iliocapsularis has also been proposed to function as a stabilizer of the anterior joint capsule. If this is true, the muscle would prevent anterior translation of the femoral head and maintain joint stability during hip extension. However, EMG activity for it was minimal during hip extension from the neutral position. Lawrenson et al. suggested that the iliocapsularis could still provide stability in other regions of the capsule.

**Surgical Relevance**

Owing to its location, the iliocapsularis is used as a surgical landmark in many procedures. During a modified Smith-Peterson approach to a Bernese periacetabular osteotomy for hip dysplasia, the iliocapsularis is used to expose the anteromedial aspect of the hip joint capsule. Elevation of it allows for entrance into the correct interval between the iliopsoas tendon and the hip capsule. The iliocapsularis is also used to identify the iliopsoas tendon during tenotomies from either the anterolateral or lateral directions. During complete hip arthroplasty, the lateral aspect of the iliocapsularis is commonly used as the location for capsulotomy. Using the intermuscular space between the inserting fibers of the gluteus minimus and the iliocapsularis as landmarks ensures a precise T-capsular incision while avoiding injury to these muscles and tendons and to the vascular supply to the hip.

**Imaging**

The iliocapsularis can be visualized by both magnetic resonance imaging (MRI) and ultrasound; however, clinicians rarely recognize it as a structure distinct from the iliacus muscle. To visualize the iliocapsularis with ultrasonography, the patient should be in the supine position with an externally rotated hip. The ultrasound transducer should be placed inferior to the anterior inferior iliac spine proximally to the anterior joint capsule inferiorly. This region marks the origin of the iliocapsularis. By moving the transducer caudally, the insertion onto the lesser trochanter is visualized. The iliocapsularis is best visualized with MRI using axial slices through the femoral head. It lies just superficially and laterally to the joint capsule. The rectus femoris is visualized superficial to the iliocapsularis. As Haefeli et al. discuss, the size ratio between these two muscles can be used to identify borderline hip dysplasia.
Conclusion

The iliocapsularis is a constant muscle on the anterior aspect of the hip joint capsule. It is believed to stabilize the hip joint. Hypertrophy of the iliocapsularis with hip dysplasia supports this belief. It could also be involved in preventing synovial impingement as the articularis genus does in the knee. Regardless of function, the iliocapsularis is used a key landmark in multiple surgeries including hip arthroplasty, iliopsoas tendon tenotomy, and Bernese periacetabular osteotomy. It can be visualized in both ultrasonography and MRI although clinicians rarely distinguish it from the iliacus muscle.

References


Correspondence to: R. Shane Tubbs, PhD, PA-C, MS, BS
Seattle Science Foundation, 550 17th Avenue,
Suite 600, Seattle, WA 98122, USA
Phone: +1 206 732 65 00
e-mail: shanet@seattlesciencefoundation.org

Conflict of interest statement: No conflicts declared.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported (CC BY-NC-ND3.0) Licence (http://creativecommons.org/licenses/by-nc-nd/3.0/) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Please cite this article as: Mashriqi F, D’Antoni AV, Wilson C, Unno F, Lee CB, Mayo K, Tubbs RS. The functional and surgical relevance of the iliocapsularis muscle: an anatomical review. Anatomy 2017;11(3):146–148.
Hypertrophy of the transverse ligament caused by os odontoideum: a case report

Seda Avnioğlu¹, Vaner Köksal², Tolga Ertekin³

¹Department of Anatomy, Faculty of Medicine, Alanya Alaaddin Keykubat University, Antalya, Turkey
²Department of Neurosurgery, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize, Turkey
³Department of Anatomy, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

Abstract

Os odontoideum is a small oval or round shaped bone fragment observed as the most common congenital anomaly of the odontoid process. Since it causes a slowly increasing mobility or instability in the atlanto-axial joint, sudden death, tetraplegia, chronic neck pain, headache, torticollis and myelopathy may develop. The spinal canal widens initially at the craniocervical junction and symptoms can occur at a very late stage. In this case, clinical complaints emerged after the age of 35. There was no other neurological finding and no history of recent trauma in this case presenting with severe neck pain. This case report shows how ligaments, the supporting connective tissue elements, can be thickened to maintain stability when the stability of the cervical spine is impaired. Since it may cause sudden death even when asymptomatic, it is important to recognize this pathology and to distinguish os odontoideum from odontoid fractures in trauma patients.

Keywords: anatomy; atlantoaxial dislocation; hypertrophy of the transverse ligament; instability; os odontoideum

Introduction

Os odontoideum (OO) is a craniovertebral junction (CVJ) abnormality in which an ossicle (small bone) is cranial to a hypoplastic dens by a variable gap. This abnormality can result in instability, which may be reducible or irreducible. What leads to irreducibility in OO is unclear.¹⁰

OO was first described as a postmortem finding by Giacomini in 1880.¹¹–¹³ Odontoid process of the axis has many variations that may either be congenital or acquired and lead to mild or severe instability of the atlanto-axial joint leading to potential defects on the medulla or upper cervical cord with neurological symptoms.¹¹ The identified congenital anomalies of the odontoid process are aplasia, hypoplasia, duplication, condylus tertius, ossiculum terminale (os avis), and OO.⁹¹⁰ OO is the most common anomaly of the odontoid process.⁹¹⁰

The etiology of OO remains controversial, but there is emerging consensus on both the traumatic etiology and a congenital source. Some studies reported that post-traumatic displacement or an unrecognized fracture of the odontoid process may result in OO prior to ossification.¹⁰ There is a progressive spondylolisthesis between C1 and C2 such as traumatic spondylolisthesis caused by hangman type fractures of the second cervical vertebra.¹¹ Therefore, trauma at etiology is often considered. Some studies suggested that OO may be a result of congenital segmentation anomaly due to failure of fusion of the ossiculum terminale.¹⁰,¹¹ Hypertrophy of the transverse ligament has been shown to maintain of the biomechanical stability, but the spinal channel is narrowed.

In this case report, we aimed to demonstrate a case report with hypertrophy of the transverse ligament caused by OO and emphasize that changes in the supporting connective tissue anatomy prevents instability in the cervical spine.

Case Report

A 35-year-old female patient was admitted to the Department of Neurosurgery, Faculty of Medicine, Recep
Tayyip Erdoğan University, Rize, Turkey with the complaint of increased persistent pain in the back of the neck for two years. The patient did not have any complaints in using his hands and in his walk. Neurological evaluation revealed no pathological reflexes or motor defects. In addition, there were no any trauma, accident, fall or bump in the history of the patient.

Coronal, sagittal and axial computed tomography (CT) images demonstrated that the apical portion of the odontoid bone was already separated (Figures 1a–c). Initial magnetic resonance imaging (MRI) revealed anterior spinal cord compression at the CVJ due to hypertrophy of the transverse ligament, which is located posterior to the odontoid bone and is responsible for its movement (Figures 1d and e). We identified the pathology as OO. Stabilization surgery was performed to connect C1 and C2 bones through the posterior neck in order to prevent serious neurological damage that could develop in the patient. The patient was followed up for four years after the surgery (Figures 2a–c). Neck pain completely improved and neurological problem was not observed until this date.

Discussion

OO can be divided into two main types: dystopic and orthotopic.\textsuperscript{[2,13]} Anomalies of the odontoid process are not observed frequently and are usually noticed after trauma and sometimes in randomized examinations. They may be associated with Down syndrome, Klippel-Feil spectrum, Morquio syndrome, and spondyloepiphyseal dysplasia. The caudal portion of the odontoid process normally completes its development, which is comprised of two lateral ossification centers.\textsuperscript{[14]} However, it may not combine with the body of the second cervical vertebrae at the level of the neurocentral synchondrosis. Since there is no bone defect at the level of the growth plate, it is also thought that OO is actually formed by non-union of a long-term and unrecognizable fracture.\textsuperscript{[6,8,15]} It has been found in some cases that the atlantoaxial dislocation occurred after trauma.\textsuperscript{[6,8,16,17]} Some studies reported that the odontoid process normally developed, but could not combine with the body of C2 due to abnormal mobility.\textsuperscript{[9,18]} It must be completely combined with the body of

![Figures 1](https://www.anatomy.org.tr)
Hypertrophy of the transverse ligament caused by os odontoideum

C2 between the ages of 5 and 7.\textsuperscript{[8,9,18–21]} Associated with this, symptoms usually occur due to atlantoaxial instability and cord compression. If accompanied vertebral artery compression is present, the symptoms become more pronounced.\textsuperscript{[8]}

Many subjects with OO remain asymptomatic throughout their life and might be only discovered incidentally.\textsuperscript{[7,10–12,22]} Excessive mobility of the atlantoaxial joint causes repetitive minor trauma in the spinal cord, blood vessels and meninges and intermittent obstruction in the vertebral and anterior spinal arteries with flexion, extension or rotation of the neck. Sudden death and tetraplegia may occur. Persistent or chronic neck pain, headache, torticollis, and myelopathy may also occur due to instability between C1 and C2 associated with OO and even the development of dislocation.\textsuperscript{[1,8,12]} Brown-Sequard syndrome caused by partial compression of cervical spinal cord may also be observed.\textsuperscript{[22]}

Fielding et al.\textsuperscript{[15]} described 35 patients with OO and detected radiographic instability in 27 of 35 patients. They performed successful posterior C1-C2 internal fixation and fusion to 26 of 27 patients with instability (Gallie type). Dai et al.\textsuperscript{[2]} described 44 patients (33 men and 11 women) with OO between ages 7 and 56 years. 26 patients had no any history of trauma. Clinical examination of the patients revealed both myelopathy and radiculopathy in 22 patients. Spiereings and Braakman reported a series of 37 patients with OO. Flexion-extension cervical spine radiograph or computed tomography was performed. They concluded that the degree of C1-C2 instability was incompatible with neurological status and myelopathy grade in 21 of 37 patients.\textsuperscript{[24]}

Since the spinal canal is wide at the CVJ in patients with OO, symptoms may appear at a very late stage.\textsuperscript{[6]} In this case, the spinal canal width was significantly reduced because the transverse ligament of the atlas, which holds the odontoid process, was hypertrophied. However, the patient still tolerated it neurologically. It is known that connective tissues (ligaments) support correct alignment of the bones and joints of the body. It has been reported that if there is a deterioration of stability or a defect in the bone roof, connective tissue can undergo hypertrophy on the ligamentum flavum and the posterior longitudinal ligament.\textsuperscript{[24–27]}

Consequently, this pathology which may cause sudden death should be distinguished from odontoid fractures in trauma patients. Therefore, the anatomy of the cervical spine bones and ligaments holding them together must be well known. In cases with OO, MRI should not be sufficient and also 3D computed tomography (CT) imaging should be performed to check whether it is fractured. We think that surgery should be decided after distinguishing features are determined by dynamic direct radiography or dynamic MRI for acute or initial instability.

Figure 2. (a, b) OO stabilized with pedicle screws on post-operative view with X-ray. (c) Post-operative axial computed tomography at the level of the C1 total laminectomy. Spinal cord decompressed with C1 total laminectomy. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]
References


Conflict of interest statement: No conflicts declared.
Introduction

The sigmoid colon, also named the pelvic colon, is the continuation of the descending colon that begins at the left pelvic brim and ends at the rectosigmoid junction in front of the third sacral vertebra. It normally lies in the lesser pelvis and is about 40 cm in length. The sigmoid loop may arise out of the pelvic in the abdominal cavity and lie in contact with loop of ileum. The sigmoid colon forms a mobile loop which lies in the lesser pelvis, but its length and form are the most variable of all colonic segments. It may remain folded and principally in contact with, or adherent to, the peritoneum overlying the iliacus muscle, or it may cross the pelvic cavity between the rectum and bladder in males, or the rectum and uterus in females, and it may even reach the right pelvic wall. The position and shape of the sigmoid colon may vary according to the length of the colon, the length and mobility of its mesocolon, the degree of distension, the condition of the rectum, bladder and uterus. Sigmoid colon morphology also depends on age, gender, geographical variation, diet, or defecation habits.

The length and diameter of the sigmoid colon vary in different races. Longer sigmoid colon and mesocolon were reported in African population, which was attributed as one of the causes of sigmoid volvulus. The length of the sigmoid colon increases with age, and males have longer sigmoid colon than females. Studies have shown that variation in the shape of mesocolon can alter the morphology of sigmoid colon.

Variations in the position and length of the sigmoid colon may produce difficulties in radiological diagnosis and instrumentation. They may also form volvulus or result in some other functional disturbances in the neighboring structures.

The sigmoid colon is a site for many acquired pathological conditions and congenital anomalies like carcinomas, volvulus, colonic varices, diverticulosis, ulcerative colitis, epiploic appendices, diverticulitis, congenital megacolon, atresia and duplication. Knowledge of the variation in the morphology and position of the sigmoid colon is of value to clinicians, pathologists, surgeons and radiologists for interventional procedures, preventing misdiagnosis and treatment of an abnormally positioned sigmoid colon.

An abnormally positioned and morphologically variant sigmoid colon: case report

Dawit Habte Woldeyes, Abebe Ayalew Bekel, Shibabaw Tedila Tiruneh, Yibeltal Wubale Adamu

Department of Human Anatomy, College of Medicine and Health Sciences, Babir Dar University, Babir Dar, Ethiopia

Abstract

The sigmoid colon is about 40 cm in length and begins below the pelvic inlet and ends at the rectosigmoid junction. It normally lies in the lesser pelvis. Anatomical variations of the sigmoid colon were described by various authors. The length and form of sigmoid colon are the most variable of all colonic segments. In this case, the sigmoid colon was found covering the left half of the transverse colon, hiding the spleen and in contact with the left lobe of the liver. In addition, it had no visible taeniae coli and sacculations. The sigmoid colon was 66 cm in length, 7 cm in lumen width, and in suprapelvic position. Awareness of the possible variation of colon especially of the sigmoid is necessary for adequate clinical, surgical and radiological management.

Keywords: length; morphology; position; sigmoid colon; variation

Sigmoid colon morphology varies with geography and race; therefore, the main objective of the present paper was to report a morphologically variant sigmoid colon that was not reported earlier in Ethiopia that was observed in a male cadaver dissected in routine gross anatomy course. Possible clinical and functional implications of the anomalous positions of the sigmoid colon were discussed.

**Case Report**

During routine dissection of a formaldehyde fixed cadaver for undergraduate medical students in the Department of Human Anatomy, College of Medicine and Health Sciences, Bahir Dar University, Bahir Dar, Ethiopia, an unusually placed and morphologically different type of sigmoid colon was observed in a 45-year-old male Ethiopian cadaver. The hospital of the College of Medicine and Health Sciences reported that the cause of death was not related to the detected anomaly. Past medical history indicated no pathological processes related to the digestive system, no traumatic injuries of the region, and no previous surgical interventions.

The dissection was performed, as regularly, according to the Cunningham’s Manual of Practical Anatomy, Volume II for 1st year preclinical medical students in the College of Medical Sciences, Bahir Dar University. With the body placed in supine position, the anterior abdominal wall was dissected and after the reflection of the anterolateral abdominal wall and removal of the peritoneum, abdominal organs were observed for their locations in the abdominal cavity and the following new features were noted (Figure 1). The sigmoid colon was found covering the left half of the transverse colon anteriorly, it also hided the spleen and was in contact with the left lobe of the liver. Normally, the sigmoid colon is expected to be located in the pelvis below the pelvic brim. In addition, the sigmoid colon had a smooth external wall - no visible taeniae coli - no sacculations as evident in other parts of the colon. We also observed that it also had a thin wall and wide lumen measuring 7 cm, especially in the middle part of it (Figure 2). The length of the sigmoid colon was 66 cm; in classical textbooks, its length is usually described as 40 cm.

**Discussion**

Anatomy textbooks describe the sigmoid colon as a visceral organ with constant morphology, but with variations in its length. Various authors reported the variant morphology of the sigmoid colon. Appropriate knowledge of these variations other than those quoted in classical textbooks is important from both medical and surgical aspects.

Madiba and Haffajee classified the sigmoid colon into three main types: classical, long- narrow and long- broad type. The classical type of sigmoid colon is the one that lies close to the pelvic brim with the normal anatomical textbook description. In the long-narrow type, sigmoid colon is long with the root of mesocolon being narrow; in the long-broad type, though the sigmoid colon is long, the mesocolon is broad.

---

**Figure 1.** Sigmoid colon taken in suprapelvic position, in contact with the transverse colon. [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]
In our case, the sigmoid colon was found covering the left half of the transverse colon, hiding the spleen and in contact with the left lobe of the liver; in addition, it had a wide lumen, and no visible teniae coli and haustra. This report matches with the long-narrow type description of Madiba and Haffajee. Madiba and Haffajee also classified the sigmoid colon in reference to the pelvic brim as pelvic and suprapelvic positions; our case falls in to the category of suprapelvic type.

Nayak et al. reported a case of sigmoid colon where it occupied the median position in the lesser pelvis, completely filling the rectovesical pouch and covering the superior aspect of the urinary bladder. This report is similar to our report in that it is a misplaced sigmoid colon, but different in other morphologies: suprapelvic in this case and infrapelvic in Nayak’s case.

Textbooks and some authors in the literature refer to the average length of the sigmoid colon as 40 cm. Cunningham’s Manual of Practical Anatomy describes the length of the sigmoid colon as 16–80 cm. The length was significantly higher in males when compared with females and considerably more in Africans. In this case, we found the sigmoid colon as 66 cm in length and 7 cm in diameter. A study performed in Nigeria in 50 fresh cadavers showed that the mean length of the sigmoid colon was 50.1±1.6 cm (ranging 34.5–67.8 cm). Bhatnagar et al. measured the length as 46.6 cm, Madiba et al. as 57 cm. A study from Turkey by Atamanalp et al. measured the sigmoid colon length as 43.7 cm. No significant correlation was reported between the length of sigmoid colon and its various types in the literature.

The morphology sigmoid mesocolon in our case was dolichocholecolic, similar to the study of Atamanalp et al., Akinkuotu et al., and Bhatnagar et al. (Figure 1). In our case, the sigmoid colon had a smooth external wall with no teniae coli and haustra; but we did not find any literature with similar morphology for further comparison.

The primary ontogenic factors responsible for positional variations of the colon are the differential development of abdominal organs with their peritoneal coverings and a mechanical factor. During retraction of the intestinal loop (large intestine) into the abdominal cavity, the dorsal mesenteries of the ascending colon and descending colon shorten and fold, bringing these organs into contact with the dorsal body wall where they adhere and become secondarily retroperitoneal. The transverse colon and the sigmoid do not become fixed to the body wall, but remain as an intraperitoneal organ suspended by mesentery. However, the sigmoid mesocolon is very long, so that it is a mobile part of the gastrointestinal tract contributing to its positional changes.

**Conclusion**

Knowledge of variations of the position and morphology of sigmoid colon contribute to the existing information of the variations in the colon and success of surgical, invasive and radiological procedures of this area. Variations in this region should not be overlooked, because these anatomical findings can help in understanding the disease process related to the colon. Since sigmoid morphology varies with geography and race, further studies on the sigmoid colon should be performed in Ethiopia.
References
A unique muscle bridge between sternohyoid and sternothyroid muscles

Murat Çetkin¹, Mustafa Orhan², İlhan Bahşi², Piraye Kervancıoğlu²

¹Department of Anatomy, Faculty of Medicine, Istanbul Medeniyet University, Istanbul, Turkey
²Department of Anatomy, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Abstract
During cadaveric dissection, a unique muscle bridge on the left anterior cervical region of male cadaver was detected and morphometric measurements were performed. The unique muscle bridge was between the sternohyoid and sternothyroid muscles. Ipsilateral sternohyoid muscle started from the lower back surface of the sternal extremity of the clavicle and from costoclavicular ligament, but it had no fibers starting from the sternum. Three nerve branches were observed separating from ansa cervicalis on the lateral of that muscle bridge; the top and the lowest nerve branches went to the sternothyroid muscle, the median branch innervated the muscle bridge. As a result of the thorough literature review made, no case similar to the variation detected in this study was encountered. During the surgical interventions such as tracheostomy, thyroidectomy and trachea resections, knowing the presence of this muscle bridge can reduce the iatrogenic injuries to this structure.

Keywords: infrahyoid; muscle bridge; muscle variation; sternohyoid; sternothyroid

Introduction
Infrahyoid muscles consist of four pairs of muscles, as superficially located sternohyoid and omohyoid and deeply located sternothyroid and thyrohyoid muscles. These muscles are responsible for movements of the hyoid bone and thyroid cartilage during talking, swallowing and mastication. Infrahyoid muscles develop from hypaxial divisions of cervical myotomes and can have variations during their embryologic development.²,³ The sternohyoid muscle orginates from the posterior face of sternal end of clavicle, the posterior upper face of costoclavicular ligament and manubrium of sternum, and inserts on to lower side of the body of hyoid bone. Being shorter and wider, the sternothyroid muscle originates from the posterior surface of the manubrium sterni inferior to the origin of sternohyoid muscle and posterior edge of the cartilage of the first rib and attaches to the oblique line on the lamina of the thyroid cartilage.² Infrahyoid muscles are innervated by the branches separating from ansa cervicalis.¹,²,³

There are variations related to sternohyoid or sternothyroid muscles in the literature.¹⁴–⁶ Sternohyoid muscle variations may be seen as double or absent, augmented by a clavicular slip, or interrupted by a tendinous intersection.² The absence of sternal attachment of the sternohyoid muscle is more frequently found than the absence of clavicular attachment.¹⁴ As a result of the thorough literature review made, no case similar to the variation detected in this study was encountered.

Case Report
During routine cadaveric dissection carried out in the laboratory of Department of Anatomy, Gaziantep University School of Medicine, a unique muscle bridge was detected between the sternohyoid and sternothyroid muscles on the left anterior cervical region of a 33-year-old male cadaver (Figure 1a). Ipsilateral sternohyoid muscle started from lower back surface of sternal extremity of the clavicle and from costoclavicular ligament, but it had no fibers which orginated from the sternum. Some fibers headed upwards from the lateral of clavicular origin of sternohyoid muscle, proceeded upwards and inwards in the form of a muscle bridge by separating from the sternohyoid at 26.4 mm higher from the clavicular origin of the muscle, and joined
the sternothyroid from the lateral. This bridge was determined to join the sternothyroid on 30.9 mm inferior of oblique line of thyroid cartilage, which is the ending point of sternothyroid. The length of the muscle bridge between sternohyoid and sternothyroid was 15.5 mm; the width of midpoint was 5.2 mm. The width of midpoint of sternohyoid on the same side was measured 18.1 mm. The width of midpoint of sternothyroid on the opposite side was 24.5 mm. There was no variation on infrahyoid muscles on right side. Three terminal nerve branches separating from a branch of ansa cervicalis on the lateral of that muscle bridge were observed. The top and the lowest nerve branches went to sternothyroid by passing behind the muscle bridge, and the middle branch innerved the muscle bridge (Figure 1b). The caliper with the precision of 0.05 mm was used in all morphometric measurements.

Discussion
Different anatomic variations can be seen on infrahyoid muscles, depending on embryological developmental process. While, among infrahyoid muscles, the variations related to omohyoid are seen most frequently in the literature, not too many publications related to the variations of sternohyoid or sternothyroid were encountered in the literature. In some studies, the fibers of sternohyoid and omohyoid are seen to fuse.\(^{7,8}\) The omohyoid muscle is the most frequently absent amongst the infrahyoid group. Absence of one belly is more frequently observed than absence of bellies. Sometimes, the inferior belly may be doubled, with the second belly possibly arising from the coracoid process.\(^{3}\) Tripathy and Preetam\(^{9}\) defined a unique case sample in which upper belly of omohyoid on left side of the neck is in tendinous form, sternohyoid is not present on right side. Raikos et al.\(^{8}\) detected that muscle fibers separating from the lower part of sternohyoid headed towards the lateral and fused with the fibers of omohyoid muscle on the lower part of the neck. Sternohyoid and omohyoid fibers can also merge at the level of the hyoid bone on the upper part of the neck.\(^{10}\) Nayak at al.\(^{10}\) defined two-belly sternothyroids as lateral and medial. The lateral belly inserted to oblique line of thyroid cartilage. Abnormal medial belly turned into a tendinous form and crossed with thyroid artery, then inserted on the same side hyoid bone and to intermediate tendon of digastric muscle. Kang et al.\(^{11}\) detected the presence of an accessory belly of sternothyroid which hended onto the right lamina of thyroid cartilage by arising from the inferomedial of origin of the left sternohyoid and heading rightward and upwards.

Unlike the previously observed variational cases, a unique muscle bridge was determined between sternohyoid and sternothyroid muscles in this study. Embriologically developing from a muscle primordium in the anterior cervical area, the infrahyoid muscles are first divided into a superficial layer and a deep layer. The deep layer develops to the sternothyroid and thyrohyoid muscles. The superficial layer becomes the splenius spreading in the cervical region, the intermediate part of which becomes degenerated in humans and the splenius is separated into the internal and external muscles. The internal muscle becomes the sternohyoid muscle and the lower part of the external muscle becomes the omohyoid muscle that runs obliquely in the lateral cervical area.\(^{11}\) The unusual connection, which was generated between deep and superficial layers during the embryonic development, may have led to the presence of that unique variation between sternohyoid and sternothyroid.

During surgical interventions such as thyroidectomy, trachea resection and infrahyoid myocutaneous flap, the surgeon should take care into presence of this muscle...
bridge. The reason that the left sternohyoid is narrower than the right one may be that the fibers separating from the left sternohyoid build up a bridge which separates from the muscle and joins sternothyroid. This case contributes to literature by the knowing the presence of this type variation by surgeons in the operations.

References


Correspondence to: Murat Çetkin, MD
Department of Anatomy, Faculty of Medicine, Istanbul Medeniyet University, Istanbul, Turkey
Phone: +90 533 867 62 16
e-mail: muratcetkin@hotmail.com
Conflict of interest statement: No conflicts declared.

Online available at: www.anatomy.org.tr
doi:10.2399/ana.17.044
QR code:

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported (CC BY-NC-ND3.0) Licence (http://creativecommons.org/licenses/by-nc-nd/3.0/) which permits unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Please cite this article as: Çetkin M, Orhan M, Bahşi I, Kervancıoğlu P. A unique muscle bridge between sternohyoid and sternothyroid muscles. Anatomy 2017;11(3):157–159.
Acknowledgment

The Editorial Staff of Anatomy expresses their appreciation to the following reviewers who have evaluated the manuscripts for Volume 11, 2017.

<table>
<thead>
<tr>
<th>A</th>
<th>D</th>
<th>K</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdulfatai A</td>
<td>D’Antoni AV</td>
<td>Karaer E</td>
<td>Sargon MF</td>
</tr>
<tr>
<td>Abegaz BA</td>
<td>Daniel AT</td>
<td>Kendir S</td>
<td>Sasaki P</td>
</tr>
<tr>
<td>Adamu YW</td>
<td>Demir M</td>
<td>Kennedy H</td>
<td>Serindere G</td>
</tr>
<tr>
<td>Adekolunkun TA</td>
<td>Duran M</td>
<td>Kervancioğlu P</td>
<td>Shahsavari S</td>
</tr>
<tr>
<td>Adekomia DA</td>
<td>E</td>
<td>Khan AA</td>
<td>Singh S</td>
</tr>
<tr>
<td>Adelodun TS</td>
<td>Enaibe BU</td>
<td>Köksal V</td>
<td>Steele AE</td>
</tr>
<tr>
<td>Adewole OS</td>
<td>Fabrizio PA</td>
<td>Lee CB</td>
<td>Stone VM</td>
</tr>
<tr>
<td>Adigun FM</td>
<td>Faiyal M</td>
<td>Loukas M</td>
<td></td>
</tr>
<tr>
<td>Adigun OO</td>
<td>Fayne A</td>
<td>Malek A</td>
<td></td>
</tr>
<tr>
<td>Akintunde WO</td>
<td>Firat A</td>
<td>Marshriqi F</td>
<td></td>
</tr>
<tr>
<td>Arayombo BE</td>
<td>F</td>
<td>Matthews K</td>
<td></td>
</tr>
<tr>
<td>Arogundade TT</td>
<td>F</td>
<td>Mayo K</td>
<td></td>
</tr>
<tr>
<td>Anniglu S</td>
<td>Gardner B</td>
<td>Obasi KK</td>
<td></td>
</tr>
<tr>
<td>Awaal O</td>
<td>Gbadamosi IT</td>
<td>Ochieng SJ</td>
<td></td>
</tr>
<tr>
<td>Awoke DG</td>
<td>Gocmen R</td>
<td>Odineal DD</td>
<td></td>
</tr>
<tr>
<td>Aydin OO</td>
<td>Gunduz K</td>
<td>Ofusori DA</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>H</td>
<td>Ogeng'o J</td>
<td></td>
</tr>
<tr>
<td>Bahsi I</td>
<td>Hassanzadeh G</td>
<td>Oluoma SS</td>
<td></td>
</tr>
<tr>
<td>Bararestani M</td>
<td>Havash F</td>
<td>Orhan M</td>
<td></td>
</tr>
<tr>
<td>Barut C</td>
<td>Horata E</td>
<td>Oskouian RJ</td>
<td></td>
</tr>
<tr>
<td>Bejide R</td>
<td>J</td>
<td>Ozturk K</td>
<td></td>
</tr>
<tr>
<td>Bekel AA</td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bekler AY</td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bodon G</td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulut E</td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-C</td>
<td>I</td>
<td>M</td>
<td>S</td>
</tr>
<tr>
<td>Choi PJ</td>
<td>Igboke CO</td>
<td>Polat Corumlu E</td>
<td>Zdilla MJ</td>
</tr>
<tr>
<td>Collin P</td>
<td>Iwanaga J</td>
<td>Protas M</td>
<td></td>
</tr>
<tr>
<td>Cornwall J</td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cetkin M</td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cicak M</td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kadikoy M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Acknowledgment

The Editorial Staff of Anatomy expresses their appreciation to the following reviewers who have evaluated the manuscripts for Volume 11, 2017.

<table>
<thead>
<tr>
<th>A</th>
<th>D</th>
<th>K</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esat Adiguzel</td>
<td>David Kachlik</td>
<td>Erdogan Sendemir</td>
<td></td>
</tr>
<tr>
<td>Nihal Apaydin</td>
<td>S. Tuna Karahan</td>
<td>Ibrahim Tekdemir</td>
<td></td>
</tr>
<tr>
<td>Cağatay Barut</td>
<td>Piraye Kervancioglu</td>
<td>Trifon Todlis</td>
<td></td>
</tr>
<tr>
<td>Neslihan Boyan</td>
<td>Kenan Kose</td>
<td>Ibrahim Tuglu</td>
<td></td>
</tr>
<tr>
<td>Mete Ertruk</td>
<td>Zelha Kurtoglu</td>
<td>Elgin Turkoz Uluer</td>
<td></td>
</tr>
<tr>
<td>Merve Didem Eskin</td>
<td>Kaan Orhan</td>
<td>Emel Ulupinar</td>
<td></td>
</tr>
<tr>
<td>Quentin Fogg</td>
<td>Mustafa F. Sargon</td>
<td>Aysel Uysal</td>
<td></td>
</tr>
<tr>
<td>Z. Asli Ikiz</td>
<td>Levent Sarkcioglu</td>
<td>Hulya Ucerler</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tuncay Varol</td>
</tr>
</tbody>
</table>
Table of Contents

Volume 11 / Issue 3 / December 2017

(Continued from back cover)

Reviews

The atlanto-occipital joint: a concise review of its anatomy and injury
Gergely Bodon, Paul J. Choi, Joe Iwanaga, R. Shane Tubbs

The functional and surgical relevance of the iliocapsularis muscle: an anatomical review
Faizullah Mashriqi, Anthony V. D’Antoni, Charlotte Wilson, Florence Unno, Cara Beth Lee, Keith Mayo, R. Shane Tubbs

Case Reports

Hypertrophy of the transverse ligament caused by os odontoideum: a case report
Seda Avnioglu, Vaner Koksal, Tolga Ertekin

An abnormally positioned and morphologically variant sigmoid colon: case report
Dawit Habte Woldeyes, Abebe Ayalew Bekel, Shibabaw Tedilu Tiruneh, Yibelit Wubale Adamu

A unique muscle bridge between sternohyoid and sternothyroid muscles
Murat Cetink, Mustafa Orhan, Ilhan Bashi, Piraye Kervancioglu

Index

Author Index to Volume 11, 2017

Acknowledgment

Acknowledgment of Reviewers for Volume 11, 2017

On the Front Cover:

Colored images of the published articles can be found in the online version of the journal which is available at www.anatomy.org.tr
Table of Contents

Volume 11 / Issue 3 / December 2017

Original Articles

Dose-dependent effect of black mustard seeds (*Brassica nigra*) extract on the prefrontal cortex of adult Wistar rats
Tolulope Timothy Arogundade, Bernard Ufuoma Enaibe, Oluwaseun Olaniyi Adigun, Foyeke Munirat Adigun, Ismail Temitayo Gbadamosi

Single dose ketamine injection affects activation of cells in the nucleus accumbens of prenatally stressed rats
Elif Polat Çorumlu, Osman Özcan Aydın, Emel Ulupınar

The accessory obturator nerve: an anatomical study with literature analysis
Mehmet Turgut, Matthew Protas, Brady Gardner, Rod J. Oskouian, Marios Loukas, R. Shane Tubbs

Standardization of sternocleidomastoid for botulinum toxin applications
Bilge İpek Torun, Simel Kendir, Aysun Uz

The zona orbicularis of the hip joint: anatomical study and review of the literature
Alexandra Fayne, Peter Collin, Melissa Duran, Helena Kennedy, Kiran Matthews, R. Shane Tubbs, Anthony V. D’Antoni

Teaching Anatomy

Bologna Process: elective lectures and internships in medical curriculum and the role of anatomy electives
Mustafa F. Sargon

(Contents continued on inside back cover)