

REVIEW

by Professor Dr Dimitar Petkov Sivrev, MD, PhD - Department of Anatomy at the Medical Faculty, Trakia University, Stara Zagora, member of the Scientific Jury according to order No. RD-09-37/27.07.2023 of the Director of the Institute of Experimental Morphology, Pathology and Anthropology with a Museum at the Bulgarian Academy of Sciences, for the defense of a dissertation for the acquisition of the scientific degree "Doctor of Sciences" in the scientific specialty "Anthropology" (01.06.01), in the field of higher education 4. Natural sciences, mathematics and informatics, and professional direction 4.3. Biological Sciences, presented by

Associate Professor **Silvia Yanakieva Nikolova** - on the topic:

**“MEDICO-BIOLOGICAL ASPECTS OF CRANIAL SUTURES:
MICROSTRUCTURE, PHYSIOLOGICAL CLOSURE, METOPIISM ”**

Documentary compliance

Associate Professor Silvia Yanakieva Nikolova has submitted the necessary documents required by law, namely:

Application for admission to protection

An European-style professional biography

Copy of document for acquired ONS "doctor" No. 34711/03.01.2011, issued by the Higher Attestation Commission

Dissertation work

Abstract draft

List of posts related to the topic

A copy of the publications included in the dissertation

List of participations in scientific events where research results were presented

List of citations of the publications included in the dissertation

Reference to scientific contributions

Reference on the minimum requirements of IEMPAM for obtaining the scientific degree "Doctor of Sciences".

It is clear from the attached documents that Associate Professor Silvia Nikolova meets the requirements for obtaining the scientific degree "Doctor of Sciences"

General characteristics of the dissertation

The dissertation work of Associate Professor Silvia Yanakieva Nikolova is presented (together with the title page, abbreviations, table of contents, appendix and supplement) on 251 standard pages according to the classic scheme for designing a dissertations. It includes the following sections:

1. Contents – 3 pages (4th-6th)
2. Introduction – 1 page (7th);
3. Literature review - 38 pages (8^{ht}-4th);
4. Purpose and tasks – 1 page (46th);
5. Material and methods – 21 pages (47th-67th);
6. Results and discussion – 129 pages (68^{ht}-196th);
7. Summary – 4 pages (197th-200th);
8. Conclusions – 1 page (20st);
9. Bibliography – 33 pages (202nd-234th);
10. Annex – 9 pages (235th-243rd);
11. Addendum – 8 pages (244th -251st);

Necessity of the study

Establishing the molecular signaling pathways and cellular mechanisms that govern the formation, function, and closure of cranial vault sutures are fundamental to normal cranial morphogenesis. Early ossification of the cranial sutures can lead to skull deformation and disturbances in normal brain activity. The object of the study is also the regulatory mechanisms on which the normal course of the morphogenetic processes, leading to the correct development and shaping of the cranial vault, depends.

The growth of the cranial bones and the increase in the volume of the cranial cavity are associated with the growth of the brain, but reaching the normal brain size does not lead to the final formation of the sutures, although they lose their osteogenic function, and the adjacent bones of the vault fuse with each other.

The study is complicated by the fact that parts of the sutures are completely under genetic control, and other areas are also under the influence of biomechanical factors of the environment. For these reasons), although suture closure is variable and does not correlate well with age, closure of the interosseous sutures of the cranial vault (especially on the inner bony surface) can be used, along with other factors, to determine the postmortem age of the individual.

Closure of bone sutures is part of the aging process. This is used in archeology to establish the age of bone remains by determining the sequence and timing of obliteration in relation to age. Obliteration begins gradually from the inner bone surface at the age of 30-40 years, and on the outer one - about 10 years later, but unevenly.

Premature closure of the cranial sutures - craniosynostosis has for decades been accepted as a pathogenetic factor for a number of clinical syndromes, as the proportionality between the volume of the cranial cavity and the growth of the brain is disturbed. Syndromic craniostosis is associated with specific gene mutations. Their study is a basic model for determining the factors that maintain the normal state of the suture. Of interest are the molecular and cellular mechanisms that govern the morphogenesis of sutures and are the basis of their pathology, as well as growth and transcription factors.

This research is necessary because the underlying mechanisms that regulate the formation, function, and closure of cranial sutures are not fully understood. The microstructure and physiological closure of cranial sutures are explored, and the specific cranial morphology in metopism is evaluated.

Analysis of the substance of the dissertation

406 authors are cited in the "Literary Review" chapter. Both modern publications and older articles were examined, which speaks of a thorough search of literary sources on the researched topic. The chapter is 38 pages long, which is a normal volume of a "Literary Review" for the scientific degree "Doctor of Sciences".

An excellent impression is made by the presence of the "Research Abstract" at the end of this chapter, which justifies the necessity of the present study and the significance of the obtained results for theory and practice.

I believe that the **goal** is formulated very well, and the specified 6 tasks are set precisely and clearly, and the conducted research corresponds to their implementation.

In the chapter "**Material and methods**" the material used is described in detail, as the cranial series are collective and unite bone material from 8 necropolises: Kavarna, Kabile, Odertsi, Tukhovishte, Batin, Kaliakra, Trastenik and Topola. From the 318 skulls of adult individuals examined, 159 were identified as male and the remaining 159 as female. Single finds were also used - grown individuals from Vinnytsia, Troshevo and a child's skull from Kaliakra. The second source of material is the ossuary of the NVIM - Sofia. These are skulls of soldiers who died in the two Balkan wars and the First World War.

The studied cranial series are homogeneous, as they represent statistically representative samples of individuals from homogeneous groups belonging to one nationality, of the same sex, who lived in the same time period. The cranial series selected in this way are one of the largest and most representative homogeneous groups studied worldwide.

Applied methods include morphometric analysis in virtual space after generating two-dimensional and three-dimensional images of the studied material using a Nikon XT H225 microcomputer tomography system. To generate polygonal/surface three-dimensional models, the skulls were scanned with a Creaform VIU scan hand-held laser scanner after preliminary placement of markers on the cranial surface. The obtained data were mainly processed with VXelements computer software.

Classical statistical analyzes and geometric morphometrics are applied to data processing and analysis, and artificial intelligence, machine learning and data mining are used to build models with high classification and prediction accuracy.

In the chapter "**Results**" are described and presented in graphic and tabular form: the microstructure of the suture, the relationship between the physiological closure of the different types of sutures and the age of the individual, the closure of the cranial sutures and metopism, the configuration of the skull in metopism, metopism and frontal pneumatization sinus, metopism, anatomical variations and pathological conditions.

The head is richly ornamented. The results are presented in 28 tables and 71 figures, which gives clarity and facilitates their easy perception.

In the "**Summary**" chapter, within 4 pages, the most important of the research is presented. The development of an original descriptive scale for reporting the contact between the bone edges in a cross-section at the level of each of the bone layers is also indicated here, which allows the precise evaluation of the degree of closure of the bone suture. The scale is based on the observed changes in the microarchitecture of the suture during its closure- Using this scale, it is demonstrated that there is a positive weak correlation between the age of the individual and the closure of the sagittal suture.

Using machine learning algorithms, regression models were developed to determine age at death by degree of sagittal suture closure. Describing the degree of closure of the outer lamina along the sagittal suture was found to give the most accurate results, but the degree of closure of this suture was found to be an insufficiently reliable indicator of an individual's age at death.

The pooled results also demonstrate that metopism is accompanied by delayed closure of sutures, presence of supernumerary vault bones, and underdevelopment of the frontal sinus, suggesting that metopism is a complex developmental disorder.

The use of methods from artificial intelligence and mathematical modeling allow, through the construction of models, to get a correct idea of the processes that take place in the development of the skull and the phenomena that are observed during its morphogenesis.

In the "**Conclusions**" chapter, 6 conclusions are presented, which are a multifaceted reflection of the obtained results. They encompass the closure of the sagittal suture in correlation with its physiological state and in association with a reorganization of the bone structure that proceeds from the inside out but is delayed in metopic skulls. The relationship between the presence of a metopic suture, reduced pneumatization of the frontal sinus, and the presence of supernumerary bones, which is due to a general disturbance in intramembranous ossification, is also indicated.

The **bibliography** covers 378 publications, from a large period of time, which allows a more complete coverage of the results published worldwide and related to the research presented.

The "**Appendix**" chapter lists: 6 contributions of a scientific-applied nature and 6 original contributions of a theoretical nature. In the presented 32 publications related to the dissertation work, in 26 Associate Professor Nikolova is the first author and the second author in the remaining 6. The dissertation student has also indicated 35 scientific reports that are related to the topic of the study.

Conclusion

From the attached table it is clear that the candidate meets the minimum requirements for acquiring the Doctor of Science degree.

Indexes	Requirements	Indexes of candidate
A	50 points	50 points
Б	100 points	100 points
Г	100 points	120 points
Д	100 points	104 points
A sum total	350 points	374 points

The candidate's points according to indicators „Г“ and „Д“ are more than the minimum requirements of the Regulations on the terms and conditions for acquiring scientific degrees and for holding academic positions in the Bulgarian Academy of Sciences. This also increases the total sum to 374 points.

Associate Professor Silvia Yanakieva Nikolova has submitted the necessary documents, from which it is clear that she meets the requirements of the Law on the Development of Academic Staff and the Regulations for its Application for the Acquisition of the Doctor of Science Degree.

In the research in connection with the dissertation work on the topic: "**MEDICO-BIOLOGICAL ASPECTS OF CRANIAL SUTURES: MICROSTRUCTURE, PHYSIOLOGICAL CLOSURE, METOPISM**" Assoc. Prof. Silvia Nikolova used modern

objective scientific methods. The results obtained through long-term work have not only theoretical, but also important practical application related to various fields of medical science.

The research represents an original and confirmatory contribution to science, which is confirmed by the contributions indicated on pages 235-236, and proves that the candidate for the scientific degree "Doctor of Sciences" possesses in-depth theoretical knowledge in the relevant field as well as abilities for independent scientific research.

I believe that the dissertation has very valuable qualities, meets all the requirements and has important not only theoretical, but also scientific and applied importance, which is why I give it **positive assessment** and I propose to the members of the respected scientific jury to award Assoc. Prof Silvia Yanakieva Nikolova the scientific degree "Doctor of Sciences", according to the requirements of the Law on the Development of the Academic Staff and clause 8, paragraph 1 of the Regulations on the terms and conditions for acquiring scientific degrees and for holding academic positions in the Bulgarian Academy of Sciences.

05.10.2023
City of Stara Zagora

Prepared the review:



/Prof. Dr Dimitar Sivrev, DM, PhD/