Ph THESIS
ABSTRACT

STUDY OF IMAGING, HISTOLOGICAL AND IMMUNOHISTOCHEMISTRY OF CEREBRAL VESSELS FROM ELDERLY

SCIENTIFIC SUPERVISOR:
Prof. univ. dr. LAURENȚIU MOGOANTĂ

PhD Student:
ENACHE ANDREEA LORENA

CRAIOVA
2012
CONTENT

Part 1 STATE of KNOWLEDGE:

Background ........................................................................................................................................ 3
Chapter 1: CEREBRAL CIRCULATION ......................................................................................... 4
Chapter 2: HISTO-ONTOGENESIS OF NERVOUS SYSTEM....................................................... 4
Chapter 3: CEREBRAL ISCHEMIA-PHYSIONEUROPATHOLOGY OF ELDERLY 5

Part 2 PERSONAL CONTRIBUTIONS

Aim of research ................................................................................................................................ 5
Chapter 4: NEUROIMAGISTIC EXPLORING FROM ELDERLY PATIENTS...................................... 6
Chapter 5: HISTOLOGICAL STUDY OF CEREBRAL VESSELS ..................................................... 8
Chapter 6: IMMUNOHISTOCHEMISTRY STUDY OF CEREBRAL VESSELS FROM ELDERLY .......................................................... 10
Conclusions ...................................................................................................................................... 10
References

Keywords: elderly, cerebro-vascular disorders, intra/extracranial stenosis, cerebral vessels, cerebral ischemia, microvascular changes
BACKGROUND

The human brain is a complex organ and heterogenic, strictly dependent on the inflow of blood. Recent researches bring more and more, into question, the idea that the development of any neurological disorders retrieving system vulnerability more strokes increased in comparison with other systems. This assumption is valid, especially in the elderly, due to the inadequate supply of oxygen and glucose.

Therefore, the most common conditions specific to persons over the age of 65, such as stroke, Alzheimer’s disease, Parkinson disease or other neurodegenerative diseases are the result of progressive and accumulative damage to the vascular system.

The most important chapter of the brain pathology of elderly is the stroke, given the increased frequency and severity of brain disorders that it generates, being one of the leading causes of morbidity and mortality worldwide.

The stroke represents the second major cause of death worldwide, after cardiovascular disease, causing about 6 million annual deaths (WHO, 2011). Moreover, all statistics indicate a progressive increase with increasing age (Rothwell et al., 2005). There are differences in prevalence, incidence and mortality increased in Eastern Europe and Japan, respectively, compared with the low rates in Western Europe and North America (Feigin et al., 2003) – explained by differences of more risk factors associated.

STROKE is the main cause of neurological disability, not only in the United States and in Europe too, especially in the case of the elderly. About 20% of the survivors of a stroke will require medical care within three months of the initial event, involving costs amounted to about $ 65.5 billion in 2008 (Fisher, 2008).

The most common causes of cerebral vascular accidents are represented by numerous cardiovascular risk, vascular and metabolic factors, severe atherosclerosis, arterial embolism, thrombosis and occlusion of small vessels (Maas et al., 2009).
Part 1 STATE of KNOWLEDGE:

CHAPTER 1:
CEREBRAL CIRCULATION

Schematically, the cerebral circulation is structured on three levels: the first level is assigned to the two internal carotid arteries and trunk basilar, the second level is made up of an anastomotic vascular system and between the rear of the brain, forming the polygon of Willis; the third level, without anastomosis, consisting of cerebral arteries in order to ensure the vascularisation of the deep brain structures.

From the anatomical and biochemical point of view, the blood brain capillaries are in direct contact with nervous tissue, the wall being doubled to nervous tissue of astrocyticator podocytes, which forms a pericapillary sheath. The capillaries are surrounded not only by a sleeve of glial substance composed of astrocytes. This astrocitator wrapper separates the vascular elements of neural elements forming the blood-brain barrier, fully formed from the third month of gestation, with the lead role to allow direct trade between the two environments, and maintaining a constant level homeostasis.

CHAPTER II.
HISTO-ONTOGENESIS OF NERVOUS SYSTEM

Central nervous system develops in its entirety in neural ectoderm that stretches from one side of the midline on the dorsal side of the embryo (Nakatsu et. Al., 2000).

By multiplying the cells in this area, in the third week of intrauterine life is caused by a thickening of ectoderm, thus creating a underplayed structure called the plate or blade neural formed by a single layer of columnar cells, neuroepithelial.

In the 6th week of intrauterine life, the neural tube has a better microscopic differentiated structure: the intern limitant, the ependimaru zone, the remaining cells will turn into ependimaru cells, the medium would later become, gray matter, the marginal zone, consisting of neuroblast of the underlying extensions, subsequent with white substance.
CHAPTER III.
CEREBRAL ISCHEMIA-PHYSIO-NEURO-PATHOLOGY OF ELDERLY

A normal brain receives approximately 100 ml of blood per 100 grams of tissue per minute in the gray substance, and 50 ml of blood per 100 grams of tissue per minute in the nervous white substance. Ischemia is defined as a reduction of blood flow, so as to modify the normal function of nerve cells.

Brain tissue is highly sensitive to ischemia, so even short periods of ischemia to the neurons can initiate a complex sequence of events, which may eventually culminate in cell death. Different regions of the brain have different thresholds for ischemic damage to the cells; the white substance is much more resistant than the grey matter substance (Mattson et al., 2001).

In relation to age, the vascular changes include the dysfunction of micro vascular structures, as well as at the level of blood flow. With the age, the vascular layers change with loss of elasticity of vessels, endothelial lesions, inflammation, reducing blood flow and accumulation of various risk factors predispose to appearance and formation of the atheroma plates with breaking and subsequent thrombosis, leading either to embolism or occlusion of the vessel (Stary et al., 1995, Frizzell et al., 2005).

Part 2 PERSONAL CONTRIBUTIONS

AIM OF RESEARCH

The objective of this pH thesis is to compare two groups of patients: over 65 years of age versus those under 65 years of age in order to study the clinical and imaging parameters-stats and to subsequently trace the changes related to individual risk factors analyzed in a Histological and Immunohistochemistry study.

In this context, I wanted to study the hypothesis according to which, the ageing process plays a factor in the atherosclerosis - defined by reducing the vascular lumen through the formation of atheroma, which significantly reduce the intake of blood to tissues leadings to the emergence of various disorders, whose statistics show a morbidity and mortality in a continuous increase.

The main objective was to highlight the importance of the knowledge of mapping and cerebral vessels circulation, in conjunction with finding histological prognosis negative markers in elderly as compared to younger patients.
CHAPTER IV.
NEUROIMAGISTIC EXPLORING FROM ELDERLY PATIENTS

Neuroimaging can be very useful in defining the various degrees of neurological pathology. Vascular Imaging can identify the location and cause of arterial obstruction, patients with increased risk of recurrence of the stroke.

A clinical statistical study was conducted on a group of 13,431 patients, of which, approximately 46.13% of this group had the study conducted at the Clinical Hospital of Neuropsychiatry, Neurology Clinic Craiova, over a period of 5 years, between 2007-2011.

Analysis of the observation sheets led to a large number of information on pathological history, neurological diagnosis, as well as on the development of the disease.

By examining the cardiovascular and metabolic risk factors (Fig 1), they have been found to be of major importance linking them with certain parameters imagistic.

![Fig.1 Risk factors for elderly carried out in neurologic hospital](image_url)
Distribution of neurological diseases for the elderly depending on the social environment, for the 7252 men from countryside reported to the 6178 women in urban areas, was as follows:

- **ischemic strokes** were found for 5423 patients, about 40.3% of them were elderly;
- **disorders of vestibular function** of 1955 cases representing 14.55% were disorders for elderly patients;
- **hemorrhagic strokes/sub arachnoid hemorrhage** at 1382 cases, about 10.28% of the total elderly disorders.
- **other degenerative neurological disorders**- 3.82% of all elderly disorders.

Of all the elderly, only a small percentage of the elderly was recommended that Doppler ultrasound, angiography, the PhD student managing to analyze only 127 of the elderly, whose suspicion of carotid stenosis and intracranial was confirmed only in 38 cases. For the categories of age 65-74 and 75-84 years, mostly affected by the carotid artery stenosis, 15 cases were reported, and 9 cases compared with younger subjects than 65 years, 14 carotidal stenosis, which led to the neurological expression, diagnosed by ultrasonography in a period of 6 months.

Ultrasonographically, expansion and arterial endothelial integrity, as well as the measurement of the dimensions of the intima of the carotid wall average were atherosclerotic process development indices, followed by the practitioner in making these non-invasive imaging methods.

Angiographically, the view of atheromatous arteries and any damage to the vascular coagulation favorsthe activity of the coagulation factors and finally, the formation of blood clots or thrombosis with severe ischemic clinical consequences.

Of the risk factors mentioned, hypertension really played the most important role in the progression of atherosclerosis lesions, neurological clinic and sometimes fatal evolution (according to the studies of Lammie et al., 1997, Stary et al., 2000).

Research on the feedback that the metabolic syndrome will not provide the additional results of the various factors for patients with intracranial stenosis are controversial (Ovbiagele et al., 2006), however, on the basis of our study, we consider that it has played a very important role for media 46.40% who presented dislipidemia.

For our group, 0.189% is the prevalence of the elderly; however, we consider that under the conditions of a screening, the percentage will increase considerably.
Whereas a recent meta-analysis show that the prevalence of carotid stenosis greater or equal to 50% is 0.2% in men aged 50 years, raising up to 7.5% in those aged 80 years or over with slightly increased frequency in women of 0-5%.

The research of de Weerd et al. (2010) includes our percentage and presents the prevalence of carotid stenosis more severe (over 70%), being in men 0.1-3.1% versus women 0-0.9%.

CHAPTER V.

HISTOLOGICAL STUDY OF CEREBRAL VESSELS

For the Histopathological study, 24 pieces of brain tissue were collected from groups of patients- 15 deaths due to ischemic strokes or bleeding, between the ages of 65 and 84 years old and 9 deaths through various other conditions, between the ages of 45 and 85 years old. Materials and histological methods were presented in detail in the current thesis and the results obtained were noted and analyzed explicitly in line with the current medical literature.

Even for this group, we correlated etiopathogenic risk factors with the atherosclerosis phenomenon, so that there were 15 cases of all those who presented hypertension (62,5%), dyslipidemia for 7 cases (29,19%), diabetes for 4 cases (about 16,66%) – most important risk factors corroborate with medical literature.

With the advance in age, cerebral vessels change its structure, form and size with reduced brain blood flow, through the appearance of various neurological disorders, having a clinical expression of acute or chronic ischemia-induced as a result of the vascular lesions leading to the death of patients.

The changes of brain vessel consisted of the lesions of atherosclerosis, for some cases, with view of the atheroma in the large and medium vessels, and other smaller vessels ships with phenomena arteriosclerosis and intraparietal injuries and led to the disorganization of arterial wall with particular layouts: telescope view (for a case, or 4,34%), snake view (5 cases, respectively 21,73%), the listed sequence of vessels (4 cases, respectively, 17,39%), spur in the lumen (3 cases, respectively 13,04%) and the total thrombosis (for 2 cases respectively 8,69%).

Atheromatous lesions were common at the level of division of vessels or curved areas, where the stroke suffered major changes of velocity and direction of blood flow.
Hemodynamic stress buildup on a specific area and the turbulence of blood are factors that were prone to the formation of plaques in the cerebral vessels and its main smaller branches.

Following the components of the blood-brain barrier, and the relationships between endothelial and architectonic items microvessels have issued the following scientific assumptions:

- thickness of internal layer, excessive deposition of collagen fibers at this level has been a powerful issue linked to the possible reversibility of lesions (patients with a sequela or younger patients) compared with vessels found predominantly in the hyalinization vessels from elderly who have a fatal prognosis much faster;
- affecting the external layer- a phenomenon referred to as inflammatory adventitia – was the event initially appeared as a result of damage to the brain vessels from a young patient died following an ischemic stroke post cranial trauma, correlate to diabetes and hypertension diagnosed in the patient's history but this hypothesis may induce a false interpretation causing reduced statistics;
- by using the technique the staining with orcein, the changes in the internal elastic lamina (or the elastolisis) may be of particular importance in the estimation of the senescence of the arteries, in assessing the biological and chronological age;
- the assumption that sinuous vessels is increased to the arterioles in the white substance, found in people with the average age of 65 years, while deeply affected by irregularities of vascular well grow progressively with age and other risk factors associated with;
- nature and extension of the lesion depends on factors such as vascular: gravity, changes in blood flow and duration of ischemia, which destroyed the blood-brain barrier for all studied patients;
- the assumption that any micro vascular changes leads to fatal and rapid evolution is sustained by the correlation of the vascular injury with the cardiovascular and metabolic risk factors.
CHAPTER VI.
IMMUNOHISTOCHEMISTRY STUDY OF CEREBRAL VESSELS FROM ELDERLY

In addition to data from microscopy, it a study was conducted using Immunohistochemistry using three imunomarkers (CD31, Alpha-SMA and GFAP) to discover other vascular changes, in the evaluation of the mechanism of atherosclerosis and to result in the involvement of all three tunics in progression injury for all patients.

Using imunostaining with CD31-for analysis of endothelium vascular changes have revealed cracks, tears or detachment of the subendothelial layer of the internal tunic, as a result of the vascular stress strongly that disrupted the functionality of the blood-brain barrier.

CONCLUSIONS

Physiology of vascular cerebral changes is complex and involved a variety of biological processes, such as:

➢ The loss of the cerebral homeostasis – explained by increasing of the frequency of neurological disorders related to the imaging parameters. Thus, the statistics highlighted for 5423 patients stroke ischemic, 1955 cases of vestibular dysfunction, 1382 cases with hemorrhagic strokes, and other degenerative neurological disorders for a small percentage 3,82% of all disorders of the elderly. The expansion and the integrity of arterial endothelial and measuring the dimensions of the intima of the carotid wall average was viewed by Doppler ultrasound or angiography for the 127 patients, for which only 0,189% of the elderly have presented extracranial stenosis.

➢ Disruption of the blood-brain barrier with glial cell activation and the appearance of specific aspects of meningeal and intracerebral vessels, such as: telescope view (for a case), snake view (5 cases), the listed sequence of vessels (4 cases), spur in the lumen (3 cases) or total vascular thrombosis (for 2 cases).

Interactions between endothelial cells and vascular smooth muscle cells in the walls of the blood vessels were the main processes in angiogenesis, in stabilizing, reshaping and vascular function, underlined by the Immunohistochemistry and the use of three markers built microscopic vessels expression, especially in the elderly.
REFERENCES


