CYTOPHOTOMETRIC DNA ANALYSIS AND IMMUNOHISTOCHEMICAL GFAP DEMONSTRATION IN HUMAN BRAIN GLIOMAS WITH CLINICOPATHOLOGICAL CORRELATES

Thesis submitted for partial fulfilment

of M.D. degree in Neurosurgery

By

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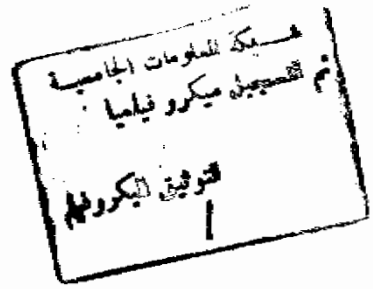
M.Sc. Degree of general surgery



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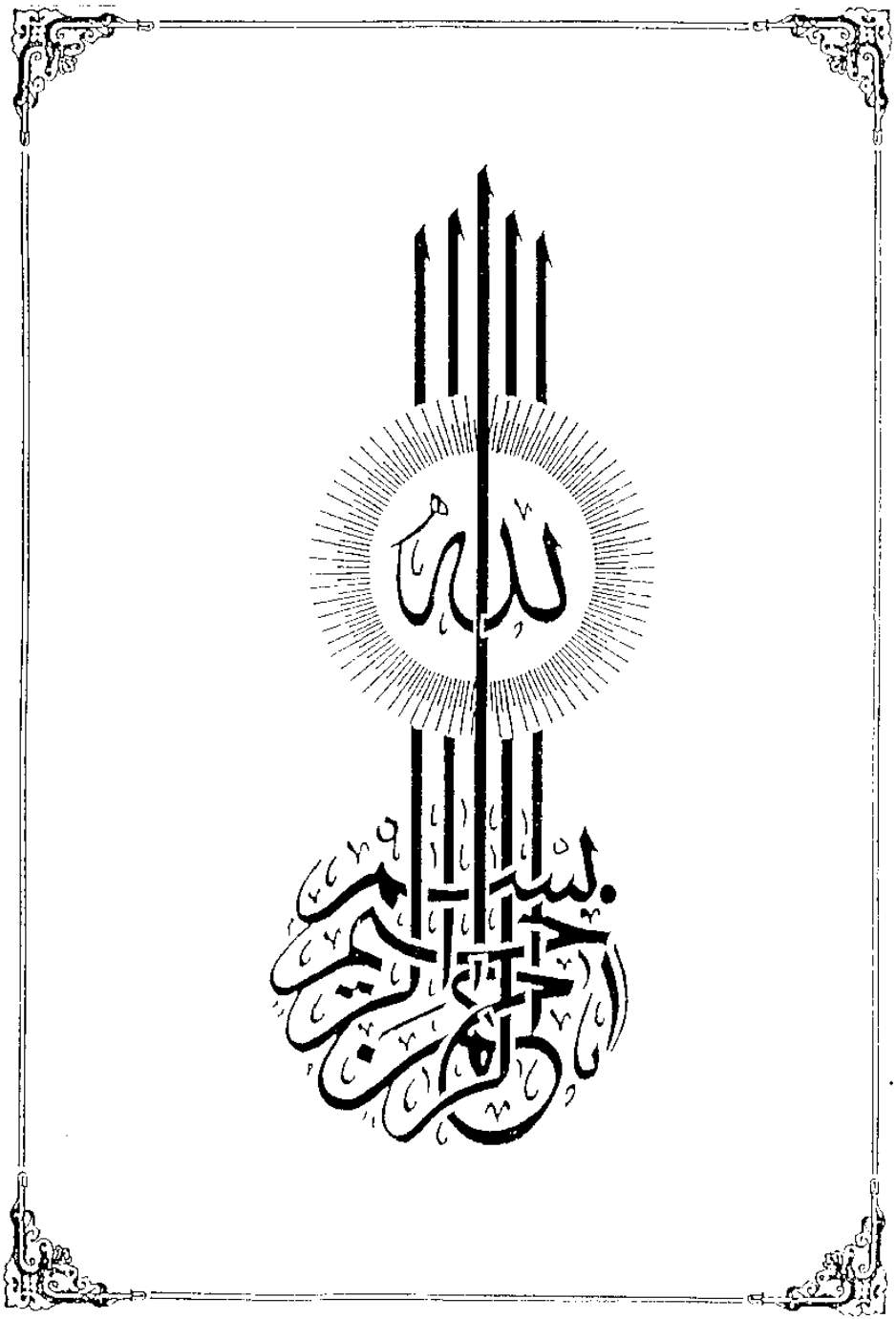
بسم ا لله الرحمن الرحيم

﴿ و الذين جاهدوا فينا لنهدينهم سبلنا،

و إن ا لله لمع المحسنين ﴾

صدق ا لله العظيم

سورة العنكبوت ، آية ٦٩



CONTENTS

To My Family

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INTRODUCTION

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Brain gliomas constitute one of the most lethal, least-controlled group of tumors [Trajanowski et al., 1989]. Not all of surgeons can do for the patient what ought to be done; sometimes, not everything that can be done should be done. On occasion, it may be best to withhold surgery altogether and proceed with palliative or supportive therapy. In the majority of cases, however, some combination of open resection or stereotactic surgery can produce a diagnosis, ameliorate symptoms, decrease the intracranial pressure (ICP), improve neurologic status, remove most of the tumor, and deliver other therapeutic agents [Salcman, 1990].

Normal human tissues contain cells with a diploid deoxyribonucleic acid (DNA) content corresponding to the normal karyotype of 46 chromosomes. The DNA content of a variety of tumor cells has been found to be greater or less than the normal diploid value. Such tumors are termed " aneuploid " . The cellular DNA content can readily be measured cytometry [Ironside et al., 1987].

Among the substances which have been biochemically defined and have varying degrees of specificity for central nervous system (CNS) tumors, glial fibrillary acidic protein (GFAP) is the best known and characterized. This molecule is widely used as an antigenic marker for normal astroglial cells and for astroglial-derived tumors. Anti-GFAP monoclonal antibodies with a high degree of specificity can now be used in the diagnosis of neurosurgical tumor biopsies and in experimental neuro-oncology [Bullard & Bigner, 1985].

Although several prognostic factors have been proposed for brain gliomas, little is known about the prognostic value of DNA ploidy and GFAP content. The goal of this study is to correlate between ploidy and GFAP of human brain gliomas and clinicopathological status of the patients in order to define the degree of dedifferentiation of these tumors and to predict the prognosis of the patients, so to know a definitive answer regarding treatment of the glioma that is currently underway.

REVIEW OF LITERATURE

PART I

PATHOLOGY

Chapter 1

GLIAL CELLS

The glial elements of the nervous system include the neuroglia (macroglia) and the microglia. The neuroglia consist of the astrocytes, oligodendroglia, and ependymal cells (Figure 1).

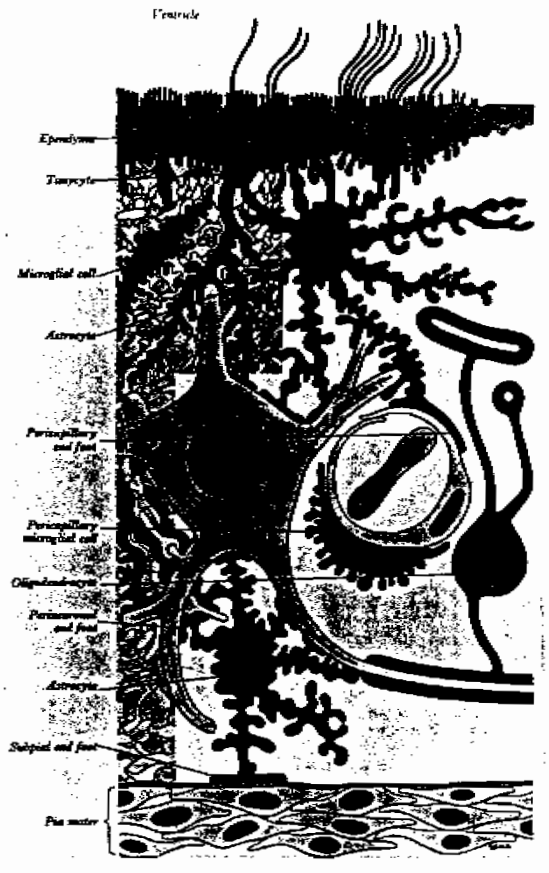

Figure 1. A schema showing the types of non-neuronal cells in the CNS. The ependymal and glial cells are shown in green. The ependyma include examples of ciliated and non-ciliated cells and one tanycyte, with a centrally directed basal process. Two astrocytes are shown apposed to a neuronal soma and dendrites; one (above) also contacts a capillary; the other (below) expands on the pial surface. An oligodendrocyte (middle right) provide myelin sheaths for two axons. Two flattened microglial cells, one adjacent to a capillary (middle right), and the other within the neuropil (top left), are also illustrated.

[Williams & Dyson, 1992]

Astrocytes

Astrocytes play a structurally supportive role in the CNS. They have been classified as protoplasmic or fibrous; the former being largely confined to gray, and the latter to white, matter (Figure 2).

Figure 2. Astrocytes. Protoplasmic astrocytes are evenly distributed through cerebral cortex. (Cajal's gold chloride X 160) [Treip, 1987]

Both types of astrocytes have rounded or oval nuclei with rather fine homogeneous chromatin. All astrocytes contain bundles of filaments, composed mainly of an acidic protein that has been characterized and purified and is known as GFAP.

The outlines of the perikarya of the two types of astrocytes differ, fibrous type having a smoother outline while protoplasmic forms having an irregular outline as if indented by surrounding structures. Fibrous astrocytes have thick long processes and rather sparse branches, many of which will be seen to attach to blood vessels. Protoplasmic astrocytes have abundant short branches that tend to form right angles.

Oligodendrocytes

Oligodendroglial cells - the myelin forming cells of the CNS - have small, dark, rounded nuclei. Their perikaryon and processes show few protoplasmic expansions (Figure 3). In the white matter these cells are seen mainly as interfascicular glia while in the gray matter they form one type of perineuronal satellite cell as well as being in relation to myelin sheaths.

Figure 3. Oligodendrocytes. These cells (center of the field) may be displayed by silver impregnation methods. The round or ovoid nucleus is surrounded by a thin layer of cytoplasm, and there are a few short processes. (Weil-Davenport X 250) [Treip, 1987]

Ependyma

The ependymal lining of the ventricles varies in appearance from place to place within the ventricular system, ranging from low cuboidal to rather taller columnar cells lining the third ventricle and its recesses. Tall cells with long tapering processes which may extend through to the outer surface of the brain are known as tanycytes, and various neuroendocrine or ionic transport functions have been speculatively ascribed to them. The ependymal cell nuclei are regularly oval or rounded. [Adams & Duchen. 1992]

Chapter 2

CLASSIFICATION AND GRADING OF GLIOMAS

Astrocytomas

Astrocytomas can be divided into three groups:

(1) fibrillary or diffuse neoplasms including the glioblastoma multiforme;

(2) pilocytic lesions encompassing the childhood astrocytomas of the visual system, hypothalamus, and cerebellum; and

(3) a large category of other neoplasms including such diverse entities as pleomorphic xanthoastrocytomas,gliomatosis cerebrei, and protoplasmic astrocytoma.

This discussion of astrocytomas deals only with the fibrillary group since these neoplasms are by far the most common and are also those for which histological grading has been best studied [Burger, 1990].

Several grading systems have been formulated for therapeutic and prognostic purposes. The first to be widely used was that of Bailey and Cushing, who likened the morphology of the neoplastic cells in these lesions to astrocytes in three stages of embryological development, namely, the astrocyte, the astroblast, and the spongioblast. Accordingly, the neoplasms which resembled these cells were named, in increasing order of malignancy, astrocytoma, astroblastoma, and spongioblastoma multiforme [Burger, 1985]. This system fell into disuse as a consequence of the astroblastoma category. The astroblastoma did not represent the majority of tumors intermediate between the astrocytoma and the spongioblastoma. The term *astroblastoma* was subsequently applied to a rare tumor entity of childhood and young adults [Burger, 1990].

The successor to the Bailey and Cushing classification was Kernohan classification. Using astrocytoma grades 1, 2, 3, and 4, this classification emphasized a loss of differentiation (dedifferentiation) rather than embryologic analogies. Increasing grades were associated with increments in the percentages of undifferentiated cells,

pleomorphism, cellularity, vascular proliferation, and necrosis. Macroscopically, the neoplasms differed in their degree of circumscription, the latter being greatest for the most malignant (Grade 4) and lowest for the well-differentiated astrocytoma (Grade1). In spite of its enthusiastic acceptance, the Kernohan system has one characteristic that makes it less than ideal: difficulty in distinguishing between astrocytomas Grade 3 and Grade 4.

One year after the publication of the Kernohan system, a simpler classification was proposed by Ringertz. By his approach, the neoplasms at the lower extreme of the spectrum are known as astrocytomas, whereas the higher-grade neoplasms are designated glioblastomas. Intermediate lesions are classified as "intermediate" neoplasms. Necrosis was the important factor distinguishing intermediate type from glioblastoma.

The classification system of the World Health Organization (WHO) recognizes four grades of neoplasms (Grades I, II, III and IV), but in effect subdivides the fibrillary or diffuse neoplasms into three grades since grade I is assigned to the pilocytic astrocytomas. The diffuse astrocytomas are classified as astrocytoma (WHO Grade II), anaplastic astrocytoma (WHO Grade III), and glioblastoma (WHO Grade IV). Because of the possibility that some glioblastomas are not astrocytic, the WHO places the glioblastoma in a separate category of embryonal and undifferentiated neoplasms.

TABLE 1

Comparison of Grading System for Astrocytomas

Bailey and Cushing	Kernohan	Rigertz	WHO
Astrocytoma	Astrocytoma Grade 1	Astrocytoma	Astrocytoma(Grade II)
Astroblastoma	Astrocytoma Grade 2	Intermediate Type	Anaplastic Astrocytoma (Grade III)
Spongioblastoma Multiforme	Astrocytoma Grade 3 Astrocytoma Grade 4	Glioblastoma	Glioblastoma (Grade IV)

[Burger, 1990]