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TAU PROTEIN AS GLIAL TUMORS MARKER: A RETROSPECTIVE STUDY

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Abstract

Tau is a known protein expressed in brain tissue. It is expressed in different neurological diseases like Huntington's and Alzheimer and also in different cancer types. Her2 protein is known to be a biomarker and prognostic factor in breast cancer and also expressed in different types of carcinoma like gastric, lung, salivary duct, ovarian and endometrial. To determine tau and Her2 protein expressions in different types of primary brain tumors and to assess the correlation between their expression and the grade of the tumor. 54 paraffin blocks of surgical specimen of different brain tumors had been reviewed, selected and categorized as glial, meningioma and pituitary tumors. Clinical details of all the cases were recorded from Immunohistochemistry department of Neurology Hospital in Baghdad/Iraq. Immunohistochemistry for tau and Her2 proteins were performed along with scoring. twenty men and 34 women were diagnosed and underwent surgery as having brain tumor: 30 cases were glial tissue, 22 cases were meningioma and 2 cases were pituitary. Tau protein distribution was as follows: 12 cases [22%] with 0 score, 24 cases [44%] were scored with + 1, 6 cases (11%) with +2 and 12 cases (22%) with +3 score. While Her2 protein expression was negatively scored in all cases. For the glial tumors a correlation between tau immunoreactivity and the grade of the tumors. This study found that tau protein is expressed in glial tumors and suggested that this protein could be a good biomarker for brain tumor classification and can be used as a confirmatory test for glial tumors diagnosis.

Key words **:** Tau protein, Her2 protein, Glioma, Glial tumors, Brain tumor.

Abbreviations: MAPT - microtubule-associated protein tau, Her2/neu - Human epidermal growth factor receptor 2, CNS - central nervous system, WHO- world health organization, FFPE - Formalin-Fixed Paraffin-Embedded, DAPI- 3.32 - diaminobenzidine tetrahydrochloride, H_2O_2 - Hydrogen peroxide, SPSS - Statistical Package for Social Science, SD - standard deviation, p- probability, FTLD- front temporal lobar degeneration, IHC- immunohistochemistry. HRP- horse reddish peroxidase.

Introduction

Globally, the incidence of most malignant brain and other central nervous system tumors is found to be low in East Asia, Southeast Asia, and India while the highest incidence have been found in Europe, Canada, the United States and Australia. This significant difference in incidence in Asian populations and European populations indicates the possible influence of origin, genetic and environmental factors in malignant brain tumors. The astrocytic tumors showed the highest distribution globally in all age groups, while medulloblastoma and other embryonal tumors were the lowest Leece *et al*., (2017).

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According to the WHO classification of brain and CNS tumors in 2016, there are many types of brain tumors including: diffuse astrocytic and oligodendroglial tumors, other astrocytic tumors, ependymal tumors , choroid plexus tumors , tumors of pineal region, embryonal tumors, tumors of cranial and para spinal nerves which include meningioma and schawnomma, and others. Glioma which is the most common primary CNS tumors accounting for 70% of primary brain tumors can be described as a collection of tumors arising from glial cells Schwartzbaum *et al*., (2006).

Glial tumors were divided into two major categories: astrocytic and oligodendroglial. They are histologically,

genetically and thus therapeutically heterogeneous. According to the WHO system, they were graded on the basis of the most malignant area identified. Grading criteria are based on the presence or absence of nuclear atypia, mitosis, microvascular proliferation and necrosis. Pathological grading of the glial tumors is essential because it determine treatment and prognosis Louis *et al*., (2016).

While, Meningioma represent approximately 20% of intracranial neoplasm, not strictly brain tumors because they arise from meningothelial cells that form the external membranous covering of the brain. Since they arise within the intracranial cavity and present with neurologic symptoms and signs, they are usually classified as brain tumors DeAngelis (2001).

During rapid tumor growth and brain infiltration, glioma cells start to secrete some signaling molecules and adhesion proteins to the extracellular matrix in order to communicate with the neighbor brain tissues to start migration and invasion if suitable microenvironments are available according to Demuth and Berens (2004).

Recently there was a discovered link between glial cells role in neuronal degeneration and tau protein. It has been suggested that as glioblastoma causes metabolic and mechanical changes stresses so it may be the cause of what is called tau neuropathology as Leyns and Holtzman (2017) studied. Tau pathology means link between glioblastoma and neurons. Tau protein expressed from the gene *MAPT* [microtubule-associated protein tau at chromosome 17, known to be involved in microtubule assembly, stabilization, neuronal morphogenesis and axonal transport mentioned by Spillantini and Goedert (2013). Any mutations or common genetic variants of the *MAPT* gene were shown to be associated with the development of tau pathology which is suggested to have a role in tau hyper-phosphorylation, aggregation and pathology (Spillantini and Goedert, 2013, Drechsel *et al*., 1992). Tau protein has been found to be expressed in metastatic breast cancer Tanaka *et al*., (2009), ovarian cancer Kavallaris *et al*., (1997), gastric cancer Wang *et al*., (2013), lung cancer Yoo *et al*., (2009) and brain tumors (Miyazono *et al*., 1993, Zaman *et al.*, 2019)

Human epidermal growth factor receptor 2, Her-2/ neu proto-oncogene, belonging to the family of tyrosine kinase growth factor receptors, is mapped on chromosome 17. It has a significant role in cell proliferation, apoptosis, cell motility and cell adhesion as defined by Fritz *et al*., (2005). HER2/neu protein overexpression and/or gene amplification have been identified in various types of malignancies including breast Fritz *et al*., (2005), colon Gluck *et al*., (2017), ovarian Tuefferd *et al*., (2007), lung Takenaka *et al*., (2011) and there were vast variations in its expression in brain tumors (Telugu *et al*., 2016, Amli *et al*., 2018)

This study was aimed to determine the role of tau and Her2/neu proteins expression in different selected types of primary brain tumors including glial, meningioma and pituitary tumors and to assess the correlation between their expression and the grade of the tumor.

Material and Methods

General clinical characteristics of human tissue samples

The study was approved by the ethics committee of Neuroscience Hospital/Baghdad/Iraq, were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Fifty four paraffin blocks of three types of brain tumors were selected from the histopathology department of the mentioned hospital, 30 of them with glioma and 22 with meningioma and with pituitary tumors.

Preparation of human tissues for immuno his to chemistry staining

FFPE (10% neutral buffered formalin, routinely processed and paraffin embedded) tissue section (3 µm thick) were cut for immunohistochemistry. De-paraffinization with Xylene-Ethanol preparation, quenching of endogenous peroxidase activity and antigen unmasking were performed by incubation with sodium citrate at about 98°C for 30 min. Using PathSitu detection kit protocols, the sections were incubated with anti-tau (A-10, Abcam, 1:50) antibody and Her2/neu (Sc-08, santacruz, 1:50) both were anti mouse IgG and HRP-conjugated secondary antibodies against mouse IgG (Sigma). The reaction products were then developed by immersing the sections in 3.32 -diaminobenzidine tetrahydrochloride DAPI solution containing 0.03% H_2O_2. The samples were analyzed using an Olympus microscope.

Quantification of the Immunostaining

Immunohistochemical staining for tau and Her2/ neu markers in the different tumors sections were scored. Immunoreactivity of tau in tumor cells was scored as follows: IHC score (0), no staining; (1+), less staining than normal epithelium, (2+), similar to normal epithelium; (3+), uniform staining more intense than normal cells. Cases with 0 or 1+ staining intensity were considered tau negative and tumors with 2+/3+ staining were considered tau positive as scored used in study of Rouzier *et al*., 2005.

Regarding Her2/neu protein the percentage of stained cells and intensity of staining were graded from 0 to 3+,

as follows: No staining (0), low intensity and incomplete membrane staining in less than 10% of cells (1+), low intensity and complete membrane staining in more than 10% of cells (2+) and high intensity and complete membrane staining in more than 10% of cells (3+). Cell staining ≤10% was considered negative (–), between 11-50% (+) and ≥51% positive (++) as mentioned in the study of Tuefferd *et al*., 2007. On the other hand, tumors with scores 0 and 1+ were considered negative, while those with scores 2+ and 3+ were considered positive as in study of Amli *et al*., 2018.

The staining results were determined by using staining intensity of normal epithelial cells as a reference and without knowledge of the clinical outcome.

Statistical data analysis

After scoring, data were analyzed using SPSS version 22. Crude data was analyzed to obtain mean and standard deviation. Chi-square was used to compare the different markers. Significant result was determined at P<0.05.

Results

In this study twenty male and thirty four female were enrolled; patients' age ranged from 21 to 57 years (mean: 40.61 ±12.19) years. Thirty subjects (55.6%) were of glial tumors types (glioma), twenty two (40.7%) were meningioma and two cases (3.7%) were have pituitary tumors as simplified in table 1.

Expression of tau in different selected primary brain tumors

Of the 54 patients, tau protein was expressed in 18 patients with glial tumors (score 2+ and score 3+) which represent 55.6% of the sample which is significant as presented in table 2-3, while meningioma and pituitary tumors were negative for tau protein, figures of immuno his to chemical staining of tau marker was shown in fig. 1. (A-D).

Table 1: Demographic characteristics and type of tumor.

Variable		No.	%
Age (years) Mean ±SD (Range)		40.61 ±12.19 (21-57)	
Gender	Male	20	37
	Female	34	63
Type of tumor	Glial	30	55.6
	Meningioma	22	40.7
	Pituitary	2	3.7

Table 2: Tau marker expression in selected primary brain tumors.

Groups	Tau 0	Tau 1+	Tau 2+	Tau 3+	P-value
Glial	3	9	6	12	0.001**
Meningioma	8	14	0	0	
Pituitary	1	1	0	0	
Total	12	24	6	12	

Table 3: Tau marker +/- expression in glioma of human brain tissues.

Tau	Glial No. / Total	Meningioma No. / Total	Pituitary No. / Total	P-value
Positive	18/30 (55.6%)	0/20 (0%)	0/2(0%)	0.001
Negative	12/30 (44.4%)	22/22 (100%)	2/2 (100%)	

Table 4: Comparison between tumor grading and scoring of glial tumors of tau marker stained tissues.

Scoring	Grading			P-value
	II	III	IV	
0	0	0	3	0.002
1+	0	6	3	
2+	6	0	0	
3+	3	6	3	

There was a significant relation between the grade of the glial tumor and the intensity of the tau protein expression represented by the score as shown in table 4.

Expression of Her2/ neu in selected primary brain tumors

Anti-neu marker expression in 54 patients with different primary brain tumors (with glioma, meningioma and pituitary tissues) were analyzed microscopically. The negative expression rates of Her2/neu was (54/54, 100%) in all patients fig. 2 and table 5.

Correlation between Expression of tau and Her2/ neu proteins in different primary brain tumors

Tau protein was positive among more than half of patients with glial tumors while the Her2/neu was negative in all patients with glial tumors. On the other hand, both markers were negative in all cases of meningioma and pituitary tumors. The scoring difference between the two markers were highly significant p value <0.0001 presented in table 6.

Discussion

Surgical biopsy represent the gold standard for glioma grading and classification. Biomarkers that provide: unique

Table 5: Percentage of Her2/ neu marker expression in glioma of human brain tissues.

Variable		No.	%
Her2neu score	Negative	54	100
	Positive	0	0

Table 6: Scoring differences between tau and neu expression markers in different brain tissue.

Type of tumor	Tau		Neu		P-value
	Negative	Positive	Negative	Positive	
Glial	12/30	18/30	30/30	0/30	<0.0001 ****
Meningioma	22/22	0/22	22/22	0/22	
Pituitary	2/2	0/2	2/2	0/2	

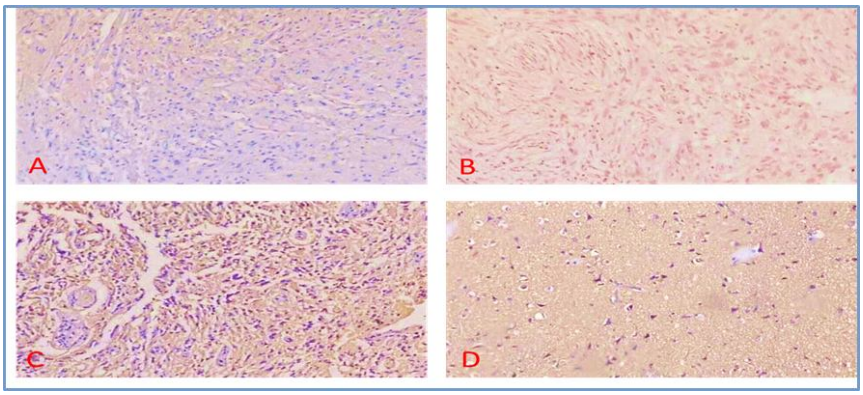

Fig. 1: Immunohistochemistry staining of tau protein expression in brain tumors, 10X magnification. A: glioblastoma cells grade IV section with score zero. B: meningioma cells with score +1 less than normal epithelium. C: anaplastic glioma cells grade III with score +2. D: oligodendroglioma tumor grade II with positive 3 score more than normal epithelium.

information about diagnosis, prognosis, needed to be highlighted for therapy mentioned by Riemenschneider (2010). Tau protein has a well-recognized role in the stabilization of microtubules, a major element of the cytoskeleton. Defective tau protein is traditionally associated with neurodegenerative disorders, such as Alzheimer's disease and front temporal lobar degeneration (FTLD). Tau protein is most commonly expressed in axons of central nervous system neurons and it can also be expressed in the somatodendritic part of neurons, oligodendrocytes and non-neural tissues Zaman *et al*., (2019).

Several studies of the relation between tau protein and cancer are reported in the brain tumor cancer literature. In past, Miyazono and his group in 1993 tried to find tau protein expression in 110 human brain tumors of patients with Alzheimer's disease and other neurological diseases. They found that tau protein positively observed in astrocytic tumors, oligodendroglial tumors, and glioblastoma, as well as neuronal tumors, but no immunoreactivity was observed in meningiomas and schwannomas (Miyazono *et al*. 1993, Simic *et al*., 2016) which is nearly similar to this study findings.

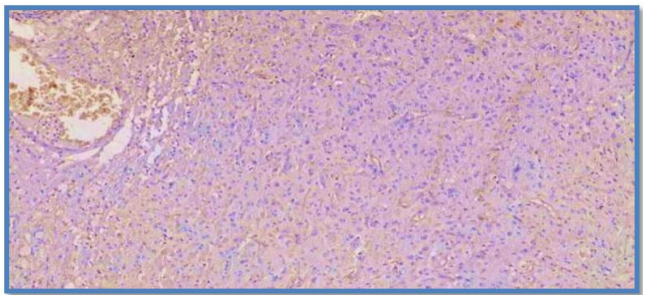

Fig. 2: Immunohistochemistry staining of Her2/neu protein marker expression in glial tumor. Negative expression with zero score at 10 X magnification.

Other study carried by Japanese group 2011 in way to determine the correlation of abnormal deposition of tau protein in the posterior pituitary gland in age related conditions. They found that the grade and the frequency of tau protein deposition were increased with aging. They give a suggestions that in posterior pituitary from elderly participants have high frequency of occurrence of deposit of abnormal tau protein in the neurons that leads to cause dysfunction of pituitary gland Hashizune *et al*., 2011.

Here, in this study there was no tau protein expression in pituitary tumors cases may be because of the young age group enrolled in the study (21-57 years).

The study in February 2019 mentioned whether tau (MAPT) expression is related to low grade glioma survival rates, they used RNASeq data representing samples from the Cancer Genome Atlas. Their study revealed that high expression of the MAPT gene is very strongly associated with increased overall and disease free survival in low grade glioma but not in breast cancer or melanoma. Also they found no such association for either amyloid precursor protein or α-synuclein gene expression. They concluded that the Tau protein may plays a neurodegenerative role and that Tau may play role in cancers of the central nervous system Zaman *et al*., (2019)

Her2/neu called as a self-protein, has role in cellular proliferation, differentiation and fetal development and was expressed in a variety of tissues of epithelial origin. In adults the Her2/neu gene presents as a single copy in normal cells. Both Her2 gene amplification and overexpression occur in various tumors including: breast, ovarian, uterine, colorectal, gastric, prostate and lung were considered as a prognostic and predictive markers in some solid tumors especially in breast tumors as defined by Papila *et al*., (2009). Then in study carried by Schwechheimer *et al*., (1994) in their attempt to determine expression of neu/c-erbB-2 in human brain tumors, they found that neu protein did not expressed in glioma but correlated with prospective relapse free internal or with the overall survival rate .

According to the recent study, they made a PubMed search on Her2 expression in neurons and meningioma cells they found its expression was variable. Glial cells were commonly neu negative, but reactive astrocytes in gliotic tissue may be positive. They suggested that HER2/

neu may serve as a significant biomarker for breast cancer. And they concluded that HER2 expression on oligodendrogliomas and ependymomas are unclear and need to establish a standardized detection system in brain tumor specimens as described by Waage *et al*., (2013). However in this study could not found any association between neu protein expressions determined by IHC and glioma tumor cells.

In conclusion, tau protein was observed to be highly associated with glial tumors and could be considered as a biomarker for this tumor category.

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Conflict of Interest: Authors state no conflict of interest.

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