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ASSESSMENT OF ZYXIN AND E-CADHERIN TUMOUR MARKER IN IRAQI PATIENTS WITH GLIOMA LESION OF THE BRAIN

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ABSTRACT : The central nervous system tumors in Iraq is consider the sixth most common tumors in adult and the second most common in child. The study included twenty six (26) cases of intracranial glioma of both gender with age range from (11 months-65 years) and all groups from Baghdad city. Formation fixed paraffin embedded (FFPE) brain excisional biopsies of retrieved from archival material of pathology laboratories of Neurosurgical hospital in Baghdad (Al-Shaeid, Ghazi, Al-Hareri Teaming hospital). Immunohistochemicaltechnique was used to detect the expression of two adhesion molecules Zyxin and E-cadherin. Distribution among age group revealed that the mean age of cases in this study was 29-93 and the median was 30 years immunohistochemical study revealed that the expression of Zyxin was expressed in 11 cases (42.3%) from all glioma cases. According to the result of the present study, we may conclude that the increasing level of Zyxin in GBM may enhance the migration of tumor cell and as a consequence increase the aggressiveness of the tumor in addition the possibility to use Zyxin as tumor marker.

Key words : Zyxin, E-cadherin, glioma.

INTRODUCTION

Gliomas are tumour of the brain parenchyma that are classified histologically on the basis of their resemblance to different types of glial cells. The major types of glial tumours are astrocytoma, oligodendroglioma, and ependymomas. The highly infiltrative or "diffuse gliomas" are the most common type (Kumar *et al*, 2013).

The gliomas as account for almost 80% of primary malignant brain tumour, so they considered the most common primary malignant brain tumour, in adults they can occur anywhere in the central nervous system (CNS) but primarily occur in the brain and arise in the glial tissue (Ostrom *et al*, 2014).

The annual incidence of CNS tumours range from 10 to 17 per 100.000 persons for intraspinal tumours, about half to three quarters are primary tumours and the rest are metastatic (Kumar *et al*, 2013). In Iraq, the CNS tumours are the sixth most common tumours in adults and the second most common in childhood (Iraqi Cancer Registry, 2012).

Meningiomas are predominantly, benign tumours that arise from arachnoidmeningiothelial cells. They usually occur in adults and are often attached to the Dura (Kumar *et al*, 2013).

Although most meningioma is easily separable from underlying brain, some tumours infiltration the brain a feature that is associated with an increased risk of recurrence (Internet, 2014).

For many tissue extracellular matrix ECM essential for the functioning of many tissues in human. At the cell levels, all cells activities such as migration, proliferation and death regulate by cell adhesion molecules.

The main feature of tumor cell the perturbed adhesion to ECM such a way that control of normal cell function is lost, this attachment is important for signal transduction from outside of cell to inside it then stimulate many activities including cell cycle progression and the cell that separate from ECM will die via apoptotic (Programmed cell death). The attachment of cell to ECM is mediated by a group of transmembrane glycoproteins that aggregate into a complex structure, which known as local adhesion (Kotb *et al*, 2018).

Zyxin is a protein (LIM domain) located to the nucleus, focal adhesion, cell-cell contact as well as a long the actin stressfiber that anchor distinct actin polymerization activity independent of the Arp 2/3 complex (Wagner *et al*, 2008). It as a proline rich domain, which may interact with slt3 domain of protein that have arole in signalling pathway (Ozkanca *et al*, 2016). Since it can shuttle between nucleus and cytoplasm so it can play a role in transcription (Grunewald *et al*, 2006; Hervey *et al*, 2006).

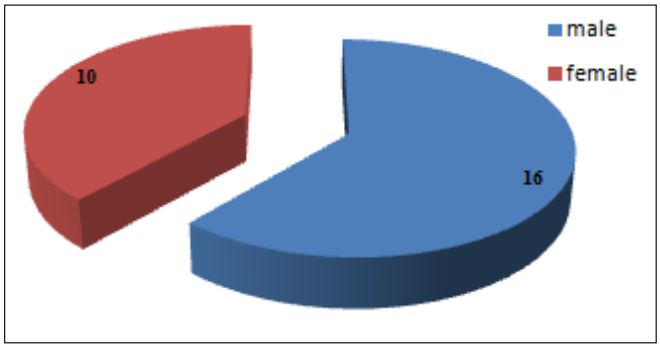

Fig. 1 : Pie chart showing distribution of cases according to gender.

Although the shuttling mechanism is unclear it more out from nucleus and this mediated by leucine rich nucleus thin export signal chain (Hervey *et al*, 2006). LIM domain are known as ducking site for many proteins, and important for many cellular function, it was found that Zyxin has a relationship with cancer development and act as possible oncogene which may promoting cancer initiation or progression, sometime it ach as tumor suppressor in other organs protecting cancer spreading (Louis *et al*, 2007), it has been suggested that Zyxin may play its role through mitotic-phosphorylation dependent manner, and with the phosphorylation action, Zyxin activity is trigger towards cancer permission rather than cancer cell invasion and metastasis (Mikheeva *et al*, 2010).

E-cadherin, which known as tumor suppressor protein, and in association with the epithelial mesenchymal transition E-cadherin expression will be lost, and this occur frequently during cancer (Cano *et al*, 2000; Yang and Weinberg, 2008; Nieto, 2011; Valastyan and Weinberg, 2011; Huang *et al*, 2012; Louis *et al*, 2007). The resulting loss of cell junction and cell-cell adhesion allow cells to separate from primary tumor and invades the adjacent tissue and move to distant sites. During EMT, the expression of mesenchymal cadherins such as N-cadherin or cadherin-11 (Lewis-Tuffin *et al*, 2010).

This research aim to elucidate the regulation of adhesive activity of two important molecules, E-cadherin and Zyxin, in the tumorigenesis and tumour metastasis. To do so immunohistochemical technique has been used to identify the expression of this two protein in a sample of Iraqi patients suffer from tumour brain (Glioma), (FFPE) formalin fixed paraffin embedded tissue.

MATERIALS AND METHODS

Patients and specimen

In aperiod from October 2017-2018, 26 cases of intracranial gliomas patients all age groups and both gender from Baghdad city, FFET brain excisional biopsies of the patients obtained from archival material of pathology laboratories of neurosurgical hospital in Baghdad (Al-Shaeid, Ghazi, Al-Hareri Teaching hosptital). All clinical diagnosis and radiological finding of site and side of affection were obtained from archival histological reports.

Histopathological evaluation

Haematoxylin and eosin stained section from each case revised concerning the pathological type and grade to prove the diagnosis of gliomas. The cases graded and classification according to WHO classification of central nervous system tumours (Lewis-Tuffin *et al*, 2010).

Immunohistochemical staining technique

Briefly, 4 ì m-thick tissue sections were deparaffinized in xylene and hydrated by immersing in a series of graded ethanol.Antigen retrieval was performed by using high pH (Hervey *et al*, 2006) at 95°C in water bathfor 40 min. Endogenous peroxidase was inhibited by added peroxide black was added and incubated for 10 min in humid chamber power block was added and incubated for 10 min humid chamber. Power block was added and incubated for 10 min in humid chamber. The primary antibody was added and incubated for 20 min, the HRP (Horseradish peroxidase) secondary antibody was added and incubated for 10 minMayer¼s haematoxylin added for 1 min as counter stain, the slides mounted by cover slides and be ready to be examined and do scoring (DAKO, USA).

RESULTS

Samples clinical analysis: During the period of three months, a total of (26) tissue samples in the form of paraffin blocks of brain gliomas specimens was included in this retrospective study. The patients' age range from (11 months-65 years), distribution among age groups revealed that the mean age of cases in this study was 29.93 and median of 30 years. There were 10 (38.46%) pediatric patients (their age less than 18 years) and 16 (61.54%) adults (>18 years). 16 cases (61.54%) were males and 10 (38.46%) were females and the male to female ratio was 1.6:1 (Fig. 1).

Histological investigation of Haematoxylin and Eosin stain (H&E) sections confirmed and grading was done to the cases according to the criteria established by WHO 2007. There were 2 case WHO grade I (Meningioma and pilocytic astrocytoma), 15 cases were WHO grade II (11 cases were diffuse astrocytoma, 4 cases were pleomorphic xanthoastrocytoma), 2 cases were WHO grade III (1 case was anaplastic astrocytoma, 1 case was anaplastic oligodendroglioma) and 7 cases were WHO grade IV (5 cases were glioblastoma and 2 cases were anaplastic Medulloblastoma (Table 1).

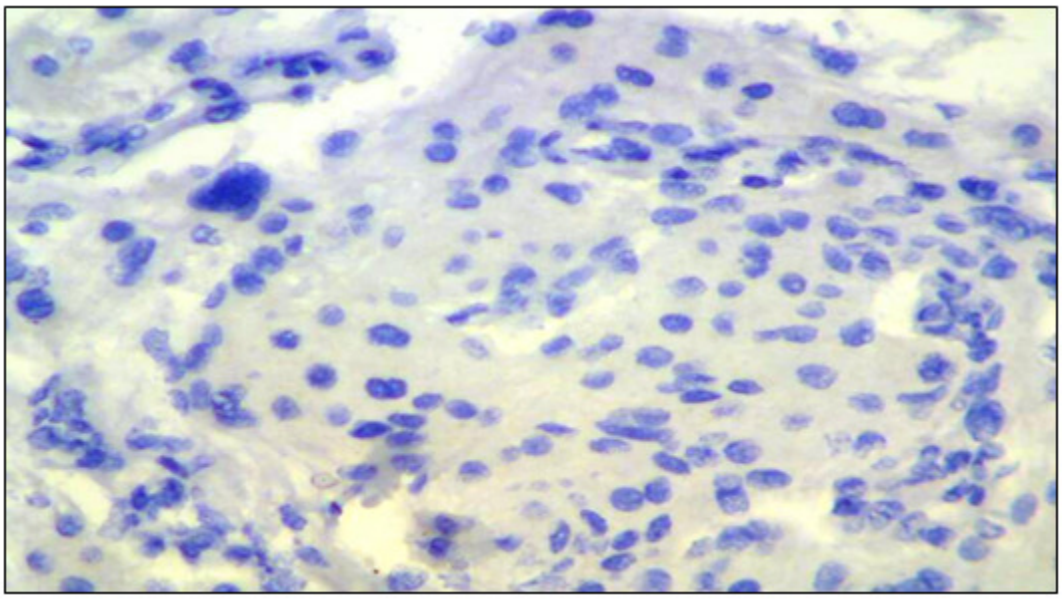

Fig. 2 : Positive cytoplasmic E-cadherin brown staining.

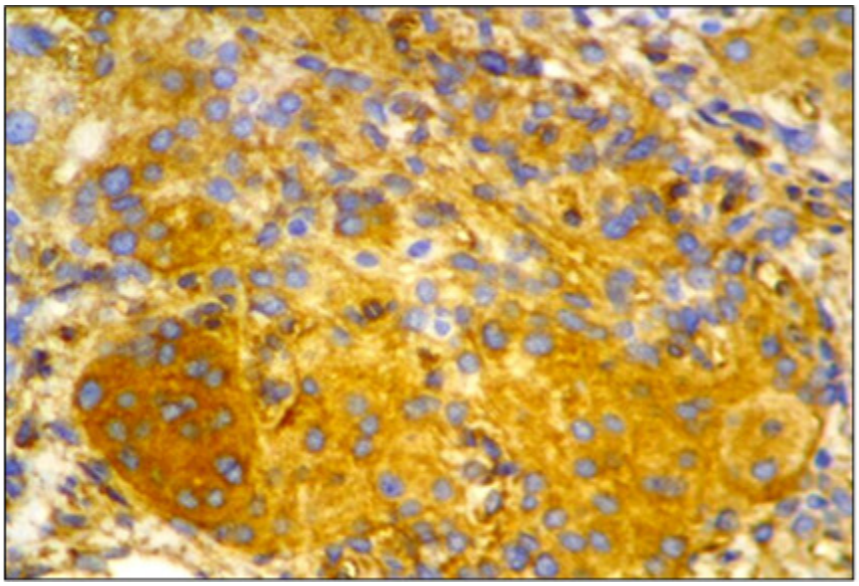

Fig. 3 : Positive cytoplasmic Zyxin brown staining.

Table 1 : Frequency distribution of different histopathological types and grade of gliomas in the studied cases.

		Types of Glioma	Count	Total
Grade	I	Meningioma	1	2
		Pilocytic astrocytoma	1	7.7%
	II	Diffuse astrocytoma	11	15
		Pleomorphic xanthoastrocytoma	4	57.7%
	III	Anaplastic astrocytoma	1	2
		Anaplastic oligodendroglioma	1	7.7%
	IV	Glioblastoma Multiforme	5	7
		Medulloblastoma	2	26.9%
Total				26 (100%)

Immunohistochemical expression of E-cadherin and Zyxin tumor marker in glioma lesions

E-Cadherin was expressed as cytoplasmic and cell membrane brown staining in the tumor cells in two cases only (2 out of 26 cases) (7.69%).

Zyxin was expressed as brown cytoplasmic staining in the tumor cells in 11 cases (42.3%) from all gliomas cases.

DISCUSSION

In the present study, expression of the representation tumour marker, Zyxin and E-cadherin was examined in a series of gliomas consisting of WHO grade I through IV tumours. Zyxin is component of focal adhesions molecules which is also convoluted in the assemblage of actin

filaments during cell migration (Drees *et al*, 1999).

Recently, it has been shown that Zyxin shuttles between the nucleus and sites of cell adhesion. In the nucleus, it binds to and inactivate the tumour suppressor protein h warts/LATSI (Hirota *et al*, 2000). In the presented work, establish an increase in the level of Zyxin in GBM relative to low-grade tumour, this agreed with Rickman *et al* (2001) and Kato *et al* (2005). Previous information have linked Zyxin to apoptosis regulation and designate both pro and anti-apoptotic activities of Zyxin, suggesting tissue and cell type specific as well as stimulus-dependent function of Zyxin (Cerisano *et al*, 2004; Chan *et al*, 2007; Wang *et al*, 2013).

Crone *et al* (2013) explain the increasing expression level of Zyxin may occur in response to sever DNA damage as a consequence to treated with chemotherapeutic drug. Another study confirmed that Zyxin was upregulatedin melanoma cells compared to melanocyte (Kotb *et al*, 2018).

Zyxin over-expression was obvious in one-third of cases and was 60 folds higher in cases with multiple tumours. LIM and Sh3 protein (LASPL) has been established to play an important role in cancer development and progression, through binding with Zyxin and affecting actin filament dynamic. Over expression is directly associated with worsening clinical prognosis and poor overall survival (Cerisano *et al*, 2004). Whether Zyxin function in GBM, to promote cell migration, inhibit h-wards/LATSI, or both, is unknown (Cano *et al*, 2000; Yang and Weinberg, 2008).

Epithelial phenotypes designated by E-cadherin expression were infrequently recognized in both low grade and high grade tumours with exception of 2 cases of diffuse astrocytomas grade II, as intuitively expected in non-epithelial malignancies (Valastyan and Weinberg, 2011). Glial tumours that absence epithelial phenotypes essentially have been observed to reorganize the cytoskeleton, dissimilar to classical EMT of epithelial tumours manifested by E-cadherin to N-cadherin shift (Grunewald *et al*, 2006; Ozkanca *et al*, 2016) similar to this data, a previous study stated that the majority of glioblastomas did not display intrinsic E-cadherin expression in aprevious study (Lewis-Tuffin *et al*, 2010). It has been a very rare occurrence to encounter malignant gliomas with E-cadherin expression (Kumar *et al*, 2013).

Malignantgliomas is prominent for biological heterogeneity and extreme fatalness, which is fairly linked with its infiltrative attribute (Kotb *et al*, 2018).

A pervious study suggested that a small population of glioma cells undergo molecular event that transport about cytoskeletal reorganization and apoptotic resistance. As a result, the tumours cells convert highly motile and invasive and then change into the treatment resistant conditions (Zhou *et al*, 2018). Compared to metastasis of most carcinomas in which the ultimate process recapitulate the organization of the primary tumours (Mikheeva *et al*, 2010), the evolutional alterations in glioma progression do not involve the organization represented by restoration of E-cadherin expression.

The current study showed that there was an opposite finding in the expression level of the two main adherent molecules, Zyxin (a focal adhesion site) and E-cadherin (Cell-cell junction site). This result may be explained as follow.

Zyxin may accumulate with high expression level in response to the DNA damage in cancer cell and the low or loss E-cadherin expression level may is a consequence for this damage (Crone *et al*, 2011).

Zyxin, a phosphoprotein, is convoluted in actin cytoskeleton assembly and is mostly localized at focal adhesion sites conserving tissue integrity. It can shuttle between cytoplasm and nucleus affecting transcription (Kotb *et al*, 2018). So this can affect transcription factor that produce E-cadherin and decrease their expression then promotingtumour progression, and invasiveness.

The finding here indicate a novel function for Zyxin in the tumor development and progression, but molecular study is required to confirm this finding.

CONCLUSION

The increasing level of Zyxin in GBM may enhance the migration of tumor cell and as a consequence increase the aggressiveness of the tumor in addition the possibility to use Zyxin as tumor marker.

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